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SYMPOSIUM

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INTRODUCTION TO SYMPOSIUM ON CANCER

WE HOPE that this symposium will bring into focus some of the more important facets of cancer investigation. No discussion can encompass research in all the broad areas of malignant disease. Particularly do we invite your attention to the important studies on cancerigenesis, which now include more and more exciting work on viruses as possible causal agents for cancer and the promising and expanding area of immunology.

Perhaps many will disagree with the attitudes and viewpoints expressed in this group of papers. We would be surprised, and to some extent disappointed, if this were not so. We feel that this symposium provides an enlightening picture of the present status of cancer research and, perhaps, insight into its future course. The reader is afforded a unique opportunity here to examine the work and the thinking of a group of outstanding investigators in such diverse fields as chemotherapy and immunology. We believe that these reviews will be of interest not only to those engaged specifically in cancer research but also to the community of medical scientists at large.

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THE NATURAL HISTORY OF CANCER

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INTRODUCTION

CANCER is a chronic disease with a long history extending back for many years before clinical signs are evident. The study of occupational cancer in man, which started with Sir Percivall Pott's recognition of chimney sweeps' cancer in 1775, and the intensive laboratory investigations of experimental carcinogenesis during the past 40 years concur in demonstrating the long time lag between the first application of a carcinogenic stimulus and the emergence of clinical neoplasia. What happens during the interim is still imperfectly known. Even under the most favorable conditions of laboratory experiment, it is difficult to follow the neoplastic process in detail from beginning to end. The natural history of cancer in man is still more difficult to trace. Apart from the formidable technical difficulties in recognizing the earliest steps in neoplasia and the successive stages in its advancement, the interpretation of accurate observations is impeded by obsolete terminologies and concepts. Histologically classifiable lesions and lumps mark conspicuous stages in neoplasia, but they imperfectly reflect more fundamental biologic changes in reactivity and behavior that determine the course of neoplasia. New information and new ideas derived from both clinical and laboratory observations open up many fresh approaches to the study of the natural history of neoplasia in man, and it is the purpose of this review to indicate possibilities of escape from some frustrations of the past.

The basic innovation is the demonstration that neoplasia is discontinuous in space and time; it is a dynamic process advancing through stages that are *qualitatively* different. The decisive steps in neoplasia are the result of *progression*, which is defined as an irreversible qualitative change in one or more of the characters of the neoplastic cells.[48] Progression alters the reactivity and potentialities of neoplastic cells; it may or may not result in visible changes in structure. An important outcome of the study of progression is the accumulation of evidence that the properties and behavior of tumors are determined by numerous unit characters, which, within wide limits, are independently variable, capable of combination in a great variety of ways, and liable to independent progression.[48,51] Lesions described as "precancerous" are visible steps in a dynamic process of neoplasia; these lesions may or may not undergo progression to a more advanced stage of neoplasia. Owing to the varied combinations of unit characters, most

of the precursor lesions, and many definitive tumors as well, are not easily classifiable by orthodox histologic criteria, and they have received various names that have done more to confuse than to clarify their real significance.

This review deals firstly with some of the experimental investigations most directly relevant to the study of the natural history of cancer and then with the stages of development of some selected forms of neoplasia in man and, more generally, with principles governing the course of neoplasia.

The Experimental Analysis of Neoplastic Development

The general principles of tumor progression have been reviewed previously.[48] This section summarizes the evidence provided by only two branches of experimental research, namely experimental skin carcinogenesis in rabbits and mice and mammary neoplasia in mice. The two branches yield complementary and overlapping information about the course of neoplasia; skin carcinogenesis illuminates, in particular, the earliest steps and mammary neoplasia the subsequent ones. Together they supply the basis for a working hypothesis applicable to the study of the natural history of human cancer.

Skin Carcinogenesis in Rabbits and Man.—Warts develop early on the tarred skin of rabbits, but some of them regress while tarring continues, and all regress if tar is withheld. Histologically, most are benign papillomas; but some, called *carcinomatoids*, are invasive and have the structure of carcinomas. The warts are *conditional* tumors whose survival and growth depend on continued extrinsic stimulation. When tar is withheld and the warts regress, the skin does not return to its original state, because renewed tarring quickly elicits warts with the same specific properties and at the same sites as those that had regressed; moreover, wound healing and some nonspecific irritants similarly promote recurrence of regressed tumors although they elicit no tumors from normal skin. On the basis of these and other investigations, Rous and his colleagues distinguished two elements in skin carcinogenesis in rabbits, namely tumor inception or *initiation* and tumor *promotion*. They inferred that initiation converted normal skin into a *subthreshold neoplastic state* characterized by the presence of *latent tumor cells*, which were stimulated to proliferate during the stage of promotion. Initiation is apparently permanent and irreversible, but promotion, in rabbits, is reversible, the skin returning to the subthreshold neoplastic state when the promoting stimulus is withdrawn. Initiating action is specific whereas promoting action is unspecific.[60,61,112,139,140]

The familiar chemical carcinogens, including tar and the carcinogenic hydrocarbons, exert both initiating and promoting actions but in varied proportions. The hydrocarbons, with a less strong promoting action, as well as initiating latent tumor cells initiate *latent neoplastic potentialities* in cells which then advance automatically but gradually and at varied, often extremely slow, rates toward the neoplastic state.[61]

In mice, similar stages of initiation and promotion have been recognized.[6,7,9] Warts develop a little later in mice than in rabbits; some of them regress, some grow for a short time as papillomas and then remain stationary, some grow progressively as papillomas, and some become carcinomas. With sustained car-

cinogenic stimulation, carcinoma develops comparatively early in almost every mouse. As carcinogenic stimulation is reduced below the optimal, the eruption of warts is delayed, and fewer of them become carcinomas. Depending on the potency of the carcinogen and the sensitivity of the mouse, a single application of carcinogen evokes either no tumors at all or long-delayed papillomas whose transformation to carcinoma is unusual. After such a suboptimal initiating stimulus, the eruption of tumors is greatly accelerated by various promoting agents, of which croton oil is the most widely used, that by themselves rarely induce neoplasia. Croton oil evokes tumors for at least 43 weeks after a single application of carcinogen, whence it is inferred that initiation is permanent. Some substances, notably urethane, seem to be pure initiating agents devoid of promoting action. These agents have been called *incomplete carcinogens;* they induce irreversible initiation but evoke no visible neoplasia unless followed by promoting agents. Most remarkably, urethane administered orally induces widespread initiation in the skin so that tumors develop at the site of subsequent applications of croton oil.[8,78,136,142] It is now questioned whether or not any of the known promoting agents is entirely devoid of initiating action, but it is clear that the promoting action is vastly predominant.

The observations on initiating and promoting phases compel a new approach to the histologic study of the mechanisms of skin carcinogenesis. General toxic and local irritative effects gravely complicate the interpretation of the changes in the skin elicited by tar. The carcinogenic hydrocarbons do not exert the most objectionable collateral actions of tar, but still their action is a complex one comprising both initiation and promotion. It has become possible only recently satisfactorily to separate these actions for histologic analysis, and much more investigation is needed. The initiating agent, urethane, even after prolonged application, produces no visible change in the skin, although the reactivity is permanently and drastically altered as shown by the eruption of tumors in response to applications of a promoting agent. Initiation, the first, most specific, and most decisive step in carcinogenesis, is accomplished without hyperplasia or any other change currently recognizable microscopically.[142]

Promoting agents elicit hyperplasia[6] but in addition exert some other action whereby they are distinguished from the generality of nonspecific irritants, which evoke a similar hyperplasia. Berenblum[7] thinks that promoting agents have two essential actions, to induce cell proliferation and to delay cell maturation. Rous and Allen[138] distinguish two kinds of promotion, namely *indirect* promotion, which brings about an environment favorable for the full manifestation of the capacities of the cells and *direct* promotion in which there is a more direct stimulus to cell proliferation as exemplified by the action of hormones on cancers of the breast and prostate. Rous and Allen think that carcinogenic hydrocarbons act both directly and indirectly, through a *dual* promotion, which, often, will be difficult to analyze. In the light of these considerations, it is probable that the early hyperplasia evoked by complete carcinogens such as benzpyrene is attributable to promoting action; it may be, nonetheless, essential to the carcinogenic action and not as Berenblum suggests an inessential side-effect. In general, carcinogenic hydrocarbons elicit, in a sensitive animal, a hyperplasia with some

distinctive features that are not usually found in the hyperplasias induced by noncarcinogenic hydrocarbons, although the correlation between carcinogenicity and histologic reaction does not invariably hold good.[69,128,141] The hyperplasia is a diffuse one. After brief application of a complete carcinogen the hyperplasia subsides, and the skin reverts to a normal appearance, but from this prepared skin, croton oil elicits a hyperplasia that differs in detail from that elicited from normal skin. The specificity of the hyperplastic response is attributable to the altered reactivity brought about by the initiating action of the carcinogen. The altered reactivity is diffuse and coextensive with the area subjected to the carcinogen.[141]

The prime difficulty in the interpretation of these observations is to account for the focal development of tumors within a wide area of tissue uniformly exposed to carcinogenic stimuli and, so far as histologic examination indicates, widely if not uniformly altered thereby. Berenblum[6,7] discounts the diffuse histologic changes as incidental and advances a hypothesis including three major propositions: (1) initiation leads rapidly or almost instantaneously to the irreversible conversion of normal cells, singly or in small groups, into latent or *dormant* tumor cells; (2) promotion depends on hyperplasia and delayed maturation of these dormant cells proceeding until a critical colony size is reached whereupon the neoplasm advances without further promoting stimuli; and (3) subsequent steps in development result from qualitative *progression*, as already defined. It has been questioned whether a few dormant tumor cells scattered through the exposed tissue can account for all the observed phenomena.[141] The early manifestations of neoplasia do not give a complete representation of neoplastic potentialities; new tumors may continue to develop within the exposed area for a long time after the withdrawal of all carcinogenic stimuli,[61,142] and there is evidence that some early tumor foci enlarge by additive growth attributable to neoplastic conversion of neighboring tissues.[69] For the purposes of the present review, it is sufficient to emphasize that neoplasia emerges focally from a wide area of exposed tissue with the reservation that the number of foci of potential neoplasia is probably large and much larger than the number of tumors that become clinically evident.

As Berenblum recognizes, neoplasia advances, after the initial disputed steps at least, by progression. Carcinoma develops rarely and late in tarred rabbits; it is usually the result of stepwise changes in a benign tumor of long standing. "What happens is no mere exaggeration of the previous papillomatosis but a wholly new event, the genesis of a neoplasm distinct from its predecessor. The changes do not always stop when a carcinoma is established; as a result of further successive changes the growth may go from bad to worse."[140] In mice, most carcinomas develop similarly by progression in a preexisting papilloma and, as in rabbits, the transition from papilloma to carcinoma represents a new step in the evolution of the tumor and is the result of a permanent qualitative change.[69] The change from papilloma to carcinoma may be abrupt or gradual. Some carcinomas develop from apparently normal skin without passing through a stage of benign papilloma.[48]

The fate of individual early "benign" lesions is not predictable. Although with optimal carcinogenic stimulation, every mouse may develop a carcinoma,

by no means every benign papilloma in any mouse develops into a carcinoma. Many papillomas regress. Shubik, Baserga, and Ritchie[150] describe how one type of benign tumor, the sessile papilloma, can behave in at least eight different ways: the papilloma may persist with unchanged characters, it may change into a pedunculated papilloma, or it may change into a conical benign tumor; it may change into carcinoma directly or through one or two intermediate stages. It is especially important to note that carcinoma can develop in several different ways, directly from apparently normal skin, from sessile papilloma directly, and from sessile papilloma through two or three intermediate lesions. It is also important to note that the "precursor" lesion is liable to extinction, to persistence without change, to change into another type of benign tumor, or to change in several different ways into carcinoma. Under optimal conditions only a proportion of the initial lesions undergo progression to carcinoma; under less favorable conditions few or none of them do so. It seems probable that the initiating stimulus determines the number of tumors that will develop and possibly also their specific characters and liability to progression whereas promoting agents determine mainly the time of tumor emergence.[149] These relationships need further investigation and are possibly more complicated than early experiments suggested.

Mammary Neoplasia in Mice.—Haaland[76] recognized successive stages in the development of mammary tumors, lung tumors, and leukemia in mice and wrote, in 1911: ". . . Intermediate stages seem to exist in which the parenchyma shows hypertrophic changes before the development of a real tumour can be proved to have occurred . . ." More recently it has become apparent that most mammary tumors in inbred mice develop from *hyperplastic nodules* like those seen by Haaland.[66,67] There are two or three different varieties of nodule, the most frequent one consisting of acinar hyperplasia. Also some tumors develop within ducts and some, apparently, from breast tissue without an intermediate stage or precursor lesion.[50,66] In some strains of mice most tumors develop from *plaques*, which being much larger than nodules are accessible to clinical as well as histologic study.[45,50] The administration of estrogens evokes widespread mammary changes in mice; when estrogen is withdrawn, as it may be without preventing the later emergence of tumors, the diffuse changes regress, and hyperplastic nodules become evident. The nodules are localized regions of specialized development in a tissue widely exposed to stimulation; the whole breast is a "field" of predisposed tissue in the sense of Willis,[172] and the nodules are specialized foci within it. The localization is not currently explicable. Carcinoma develops in some of the nodules, but the others, usually the great majority, persist as nodules or regress. Plaques, like nodules, are specialized regions of the breast, and carcinoma develops within them, usually in one or a few places, but sometimes more diffusely. In principle, the paths of development through nodules and through plaques are similar; in each there is a sequence of qualitatively different stages. The development is by progression. At each step progression occurs, most commonly, in only a portion, and usually a small portion, of the available tissue. The sequence of changes does not advance automatically and inevitably; progression can pause or halt at any stage; it may follow any one of

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a number of possible pathways, and it may vault or by-pass some of the steps in the sequence.

Many of the mammary tumors that develop from plaques are for a short or a long period of their development hormone-responsive; they grow only during pregnancy and regress promptly after parturition. The responsiveness to pregnancy is liable to be lost sooner or later by progression to an unresponsive state. This phenomenon is particularly convenient for the study of general principles of progression that are widely applicable to neoplasia in animals and in man.[45,48] The main principles are here summarized.

General Principles of Progression.—

Independent progression of tumors: It is the general rule that only one or a small proportion of multiple tumors present in the same host undergo progression at one time. The progression may be triggered by extrinsic stimuli, but it involves a qualitative change within the tumor itself.

Independent progression of characters: Different identifiable characters of a tumor undergo progression independently of one another. In mammary tumors of mice, responsiveness to hormones and growth rate are liable to independent progression and so are growth rate, histologic type, and invasiveness in chemically induced skin tumors.[150] This rule leads to the more general proposition that the structure and behavior of tumors are determined by numerous *unit characters*, which, within wide limits, are independently variable, capable of highly varied combination, and liable to independent progression. The orthodox division of tumors into benign and malignant is no longer adequate, and in many tumors in animals it is not possible. It is necessary to take into account a wide range of tumors almost infinitely graded in their combinations of growth rate, invasiveness, powers of metastasis, responsiveness to hormones, histologic type, and many other characters. A tumor may have all the cardinal signs of a malignant growth and yet be fully dependent on extrinsic hormonal stimulation for growth or even for survival.

Progression is independent of growth: Progression occurs in stationary, regressed, or dormant tumors although in some forms of neoplasia, continued growth seems to favor progression and may be essential for it. Two corollaries of this rule are that (1) at its first clinical emergence a tumor may be at any stage of progression, and (2) progression is independent of the size or clinical duration of a tumor. The abuses in the use of the term "early" are becoming recognized.[105] The early tumor, so judged by short clinical duration or small size, may be in a "late" stage of progression toward highly malignant behavior.[47]

Progression is continuous or discontinuous: It may advance slowly and gradually or by abrupt steps.

Progression follows one of alternative paths of development: An important feature of early stages of neoplasia is the choice of qualitatively different pathways of development. Several examples of the diverse fates open to lesions of one kind have been described already. The early warts evoked by chemical carcinogens and the hyperplastic nodules in the breasts of mice can regress, persist without change, or advance in several different ways. Conversely, one

lesion, for example carcinoma of the skin or of the breast, may develop in several different ways by direct paths or by indirect paths traversing intermediate stages or precursor lesions. Progression may consist of steplike advances along one path of development, or it may bring about a decisive change of path. In late stages of neoplasia, progression almost always leads to increased aggressiveness, but this is not true in early stages; at earlier stages neoplasia may stand still, regress, or be diverted to a relatively harmless path.

Progression does not always advance to an end point within the lifetime of the host: Transplantation experiments show that a tumor rarely advances to a definitive stage in its original host. In general, progression may halt at any stage; advance toward a theoretical end point is not inevitable or predictable. Some undoubted neoplasms reach "dead ends" of development, never progressing beyond sluggish benign growths, some few regress, and some, like neuroblastoma, become quiescent by a process resembling maturation.

The Natural History of Cancer in Man

A comprehensive and detailed analysis of the natural history of human cancer is outside the scope of a review article. The intent of the present discussion is to illustrate the application of general principles of tumor progression and neoplastic development to the study of human cancer. The first part of this section deals with early stages of some selected forms of neoplasia, and the second part deals more generally with the later course and manifestations. The division is an arbitrary one for convenience of presentation; similar general principles apply at all stages of neoplasia which needs studying from beginning to end as one dynamic process.

DEVELOPMENT OF NEOPLASIA

The subjects chosen for discussion in this section include neoplasia of the skin and neoplasia of the bladder, which allow the most direct application of experimental findings to human disease, intestinal polyposis, neoplasia of the uterine cervix on account of the current interest in the detection and treatment of early stages, carcinoma in situ in other situations, and finally mammary neoplasia to illustrate the application of general principles to a special case of great complexity about which much dispute and confusion persist.

Epidermal Neoplasia.—Many disorders of the skin have been called "precancerous" with varied degrees of justification. In a valuable review Bloch[11] remarks that no unequivocal definition of precancerous is at present possible. He accepts as precancerous those pathologic changes in tissues which, without being cancers, show the tendency sooner or later to develop into actual cancer, but he makes justified reservations about the difficulties of applying the definition in practice owing to lack of precise knowledge about the frequency of the development of cancer and the absence of agreement about the frequency needed to justify the diagnosis of precancerous. Bloch refers to three main groups of precancerous states or *precanceroses*. The first group includes diseases like xeroderma pigmentosum, Bowen's disease, Paget's disease, and senile keratosis which, in principle, always eventuate in cancer, although the process may be

so slow that the patient dies before cancer develops; the second group including leukoplakia, radiodermatitis, and nevi progress to cancer much more rarely, and a third group of varied lesions including lupus and scars, which change into cancer so exceptionally that it is unreasonable to call them precancerous. As Bloch notes, one type of precancerosis can develop into different kinds of cancer, and one type of cancer can derive from several types of precancerosis.

The following summary of some of the conditions in some sense precancerous shows how closely neoplasia in the human skin conforms with the general principles of progression enumerated in the preceding section. The main differences between the experimental and human diseases are in terminology. The laboratory worker identifies the start of neoplasia with the process of initiation of which there may be no gross or microscopic signs and recognizes various stages of development for which the orthodox pathologic terms are dubiously appropriate. In general, he describes as "benign neoplasia" the stage that the dermatologist calls "precancerosis." The latter term seems to me unobjectionable, provided it is recognized as one stage in a process of neoplasia whence progression to carcinoma may or may not occur. In the first of Bloch's groups, at least, the precancerosis is neoplastic, but to introduce the terms cancer or carcinoma, however qualified, creates more confusion than it removes. The several conditions next described are chosen to illustrate the varied paths of progression, and the varied associations of unit characters that defy classification by the conventional methods. The occupational or environmental neoplasias are described first on account of their close resemblance to experimentally induced neoplasia.

Chemically induced precancerosis: Neoplasia attributable to oils, tars, and pitches follows much the same course as in animals. There are minor differences in the neoplastic reactions to the various materials and some major differences in the speeds of reaction. Coal tar products act more quickly than mineral oils, the maximum incidence of tumors occurring about 20 and 50 years, respectively after the first exposure. Tumors may develop up to 40 years or more after the last exposure to the carcinogen.[80,81] Kennaway[102] finds that a much more intense carcinogenic stimulus is needed to shorten the induction time than to increase the tumor incidence. Experimental investigations suggest that the incidence depends mainly on the initiating stimulus and the induction period on promoting action.

Hueper[90] describes the cutaneous reactions in three groups: (1) acute and chronic inflammatory reactions (erythroderma, dermatitis, and folliculitis); (2) pigmentary disturbances (melanosis, leukoderma); (3) hyperplastic and neoplastic lesions (hyperkeratosis, intradermal papules, warts, pedunculated and broad-based papillomas, cutaneous horns, and carcinomas).

The lesions are commonly multiple and varied. Some papillomas remain benign or regress; others undergo progression to carcinoma. Carcinoma develops by progression in several ways in warts, papules, and ulcers; but it may develop also from apparently normal skin many years after all inflammatory and hyperplastic lesions have disappeared. After successful treatment of early growths, new tumors may develop throughout the rest of the patient's life.[81,90] One or more of the lesions may undergo progression to carcinoma. Henry[81] mentions

one patient in whom all the multiple growths were malignant. Little attention is given as a rule to the fate of particular lesions, but it seems that, in general and in conformity with the rule of independent progression of tumors, only one or a small proportion of the benign lesions or precanceroses undergo progression to carcinoma at one time[146]; if these are successfully treated, carcinomas may develop later from other lesions or from apparently normal skin. The continued emergence of tumors for many years after withdrawal of the carcinogenic stimulus implies a permanent initiation of neoplasia more widespread than indicated by the first "precancerous" lesions.

Arsenic cancer: The medicinal use of arsenic is responsible for the majority of arsenic cancers. The length of exposure extends to many years, the average being about 18, and carcinoma develops up to 18 years after withdrawal. After many years of exposure the patients develop multiple keratoses with a characteristic distribution. These lesions persist for a long time, on the average about 10 years, and then one or more undergoes progression to carcinoma, which is of either squamous-cell or basal-cell type. The carcinomas are often multiple but not numerous. Any keratotic lesion may undergo progression to carcinoma but not all of them do; some regress. Carcinoma may develop also from apparently normal skin, and it may develop by different paths in the same patient. Squamous-cell carcinoma and basal-cell carcinoma may exist together or with Bowen's disease or multiple superficial epitheliomatosis in one patient. It is noteworthy that relatively few of the patients who receive arsenic medicinally develop keratoses and still fewer develop carcinoma.[22,58,120,155]

Ionizing radiations: Ionizing radiations bring about a chronic radiodermatitis, which varies in detail according to the dose and duration of irradiation. In general, progression to carcinoma may occur, after latent periods varying from 3 to 50 years, in the margins of ulcers, in keratotic lesions, or probably in skin that is not visibly damaged.[70,90]

Senile keratosis: This, according to Bloch,[11] is the most frequent and typical precancerosis of the skin. It occurs especially on the face and to a lesser extent on the hands or on the forearms so far as they are kept bare. This condition seems to include Farmer's skin and Fisherman's skin and is possibly due to ultraviolet irradiation and identical with solar dermatitis. The entire skin of the face is almost always in a state of more or less advanced senile degeneration. Upon this skin, multiple focal lesions of keratosis develop, and sooner or later some of the lesions, usually warts, undergo progression to squamous-cell or basal-cell carcinoma. Some patients develop hyperpigmented spots, progression in which leads to malignant melanoma. It is not clear how often progression occurs or in what proportion of the lesions.[11,90,109]

Xeroderma pigmentosum: Xeroderma pigmentosum is an inherited disease transmitted as a recessive condition[114] in which the whole skin is hypersensitive to sunlight. The disease usually becomes manifest in early childhood following exposure to sunlight. The early changes in the skin include erythema and scaling and increased pigmentation in focal areas resembling freckles. Later, areas of atrophy, telangiectases, and warty keratoses develop. Histologically, irregular areas of atrophy, acanthosis, hyperkeratosis, and increased pigmentation are

found. Many of the warty papules and nodules regress after a time, but some of them undergo progression to carcinoma. Progression of one or more of the lesions occurs almost inevitably in every patient, sometimes before puberty and often during adolescence or early adult life. Rarely, progression in pigmented areas leads to malignant melanoma.[21,166] The remarkable feature of the disease is the inevitability of progression in one or more of the multiple focal lesions that develop in response to solar radiation. Bloch[11] does not consider the whole skin as precancerous but as the terrain necessary for the development of the focal precancerous lesions. Apart from questions of terminology, it is evident that the whole skin is abnormal and predisposed to neoplasia, which is probably initiated considerably before the eruption of lesions. It is noteworthy that as in mice subjected to optimal carcinogenic stimulation, carcinoma develops in almost every individual, but not in every lesion.

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Various "precancerous" lesions: Carcinoma develops on the basis of various lesions of the skin so infrequently that the designation precancerous is dubiously applicable in any useful sense. These lesions include lupus, psoriasis, varicose ulcers, and scars, especially those following burns.[11,109,172] Scars following burns are, perhaps, most deserving of inclusion as precancerous lesions. Willis remarks that the neoplastic changes in scars are often multifocal or widespread so that the whole scarred area seems to be a potentially cancerous "field." Neoplasia in areas of psoriasis is often but not invariably attributable to arsenic or irradiation used in treatment.[70,120]

Melanoma: Malignant melanoma develops in the great majority of patients from a junctional or "compound" nevus.[1] Much less commonly it originates from apparently normal skin or from pigmented areas in senile or solar keratosis[11] or in xeroderma pigmentosum.[166]

Although junctional nevi provide the origin of most malignant melanomas the great majority of them do not undergo this progression. Allen and Spitz[1] describe six fates to which junctional nevi are liable; they may (1) lie dormant, (2) regress, (3) develop into intradermal nevi, (4) develop into compound nevi, (5) develop into juvenile melanoma, or (6) develop into melanocarcinoma. There is substantial evidence that trauma can "precipitate" the progression of junctional nevus to malignant melanoma[172]; whether by a trigger action or by providing an initiating or promoting stimulus is not known.

Juvenile melanoma is scarcely distinguishable histologically from malignant melanoma, but although it is invasive it does not metastasize. At puberty it may take on the histologic characters of a simple intradermal nevus, it may persist with characters unchanged into adult life, or it may undergo progression to malignant melanoma.[1] Juvenile melanoma seems to stand between a nevus and a malignant melanoma differing from the latter in lacking the unit character upon which dissemination depends.

Bowen's disease and allied lesions: Bowen's disease takes the form of multiple papules or plaques which, histologically, are intraepithelial carcinoma of squamous-cell type with dyskeratosis. Bowen's disease is a histologic entity but probably diverse in etiology and pathogenesis.[11] The cellular characters are those of carcinoma, but the lesions are not invasive; they may persist without

change in characters for many years or, after a long period, progression to carcinoma may occur in about 20 per cent of the patients although the estimates of the frequency of progression vary widely.[11,100,109,120,172] A comparable lesion of mucous membranes undergoes progression to carcinoma more frequently.[19,160]

It is now customary to classify Bowen's disease as a carcinoma in situ. Whether or not this term is better than precancerosis is arguable. Its chief advantage is in making a distinction between Bowen's disease and other precanceroses such as keratosis. Bowen's disease has more of the characters of a carcinoma, but it is not invasive.

Extramammary Paget's disease and some examples of *kraurosis vulvae* are closely allied to Bowen's disease and similarly liable to progression.[172]

Erythroplasia (or erythroplakia) is a rare disease occurring in the mouth, on the lips, on the vulva, and especially on the glans and prepuce. The single or multiple lesions consisting of epithelial hyperplasia with dyskeratosis closely resemble Bowen's disease and deserve separate mention only because progression to carcinoma seems to occur in every patient who is kept under observation for a sufficient length of time.[109]

Multiple (benign) superficial epitheliomas: These lesions, referred to also as multiple superficial epitheliomatosis, are classified by Mackee and Cipollaro[109] as a *flat* type of basal-cell carcinoma. The lesions are multiple and usually numerous. Histologically most of the lesions resemble basal-cell carcinoma, but some are of squamous-cell or mixed types. They are distinguished from other basal-cell or squamous-cell carcinomas by their clinical behavior. Clinically they are benign; they are not prone to ulceration, do not metastasize, and persist for 10, 20, or 30 years or more. Occasionally, one portion of a lesion undergoes progression to metastasizing carcinoma.[109,120]

Self-healing squamous carcinoma of the skin: Self-healing tumors of the skin have been described as squamous carcinoma, keratoacanthoma, molluscum sebaceum, and molluscum pseudocarcinomatosa with considerable disagreement as to whether they are all the same or not.[5,17,23,31,52,75,107,115,137,151,152,154,173]

There are three groups of tumors[115]: (1) The rare multiple tumors originally described by Ferguson Smith as self-healing carcinomas. This type is characterized by multiple lesions on exposed skin histologically closely resembling, or indistinguishable from, squamous carcinoma. After a short period of growth, the tumors regress completely, leaving only scars. Local invasion occurs during the growing period but is limited as a rule to the dermis. There is no dissemination. New tumors may appear and regress successively for many years; they spring from previously normal skin and not from areas of keratosis or dermatitis. (2) In a second type of the disease, also rare, the lesions are extremely numerous, several hundreds being present at one time.[107,173] (3) In the third type, the lesions are usually solitary. This type seems to be common, accounting possibly for about one third of all squamous-cell "cancers" of the skin.[5]

There is no general agreement as to whether or not this disease is neoplastic. There is some circumstantial evidence, but no proof, that molluscum sebaceum is a virus-induced disease.[52] On the other hand, chemically induced tumors in animals provide many examples of conditional and regressing tumors including

some like the *carcinomatoids* on rabbit's skin that are invasive and histologically like carcinoma although they are conditional and regress when carcinogenic stimulation is withdrawn.[60,139] Whiteley[169] describes regression of similar invasive tumors induced by a carcinogenic hydrocarbon on rabbit's skin and specifically notes their resemblance to molluscum sebaceum. Whiteley records occasional progression of the rabbit tumors to progressively growing tumors. Clear evidence of progression in the human tumors, not yet forthcoming, would be in favor of a neoplastic process. Whatever the true interpretation may be, it is of practical as well as theoretical importance that some invasive tumors, hardly if at all distinguishable histologically from squamous carcinoma, regularly undergo spontaneous regression.

Characteristics of epidermal carcinogenesis: Recurrent features in many of the processes described in the preceding paragraphs are that progression to a "precancerosis" occurs only in focal areas of skin widely exposed to physical or chemical injury and that further progression to carcinoma occurs only in some of the lesions in some of the patients. The probability of progression from precancerosis or benign lesion to carcinoma varies widely from one group of lesions to another. Although the *usual* path of progression to carcinoma may traverse a distinctive precursor lesion there are often alternative paths through different lesions and almost always, it seems, there is an alternative direct path from apparently normal skin traversing no precursor lesion at all. In harmony with experimental findings, it is evident that initiation of neoplasia, including malignant neoplasia, can occur without visible change in the skin and far more diffusely than is indicated by the "precancerous" lesions. The precursor lesions, further, although liable to progression to carcinoma do not inevitably undergo that progression and, as a rule, most of them do not; alternatively and more usually they persist with unchanged characters, or they regress. Even in xeroderma pigmentosum, by no means every lesion undergoes progression to carcinoma although almost every patient dies of cancer.

Difficulties of terminology spring mostly from unfamiliar associations of unit characters not provided for in orthodox pathologic classifications. Bowen's disease is called precancerosis or carcinoma in situ for want of a better name; neither term is satisfactory to describe a stage of neoplasia which looks rather like a carcinoma but does not behave like one. Self-healing carcinoma, again, has the histologic characters of carcinoma but, inconsistently therewith, habitually regresses.

Neoplasia of Vesical Epithelium.—Experimentally induced tumors of the urinary bladder are varied in structure, ranging from localized intravesical papillomas of benign appearance, through pleomorphic tumors invading the bladder wall, to malignant sarcoma-like growths penetrating the bladder wall and disseminating in the peritoneal cavity. Some tumors are apparently malignant from the beginning whereas other malignant tumors develop by progression in benign papillomas. Progression does not always reach an end point in the original host; it may halt at any stage, or the host may die from hemorrhage or urinary obstruction before progression is far advanced. If progression is incomplete in the original host it advances during transplantation in normal

animals, showing that progression is not dependent on persistence of the original carcinogenic stimulus. In mice, acetylaminofluorene evokes widespread hyperplasia or papillomatosis, but early gross tumors are usually solitary and often pedunculated, developing from only a small area of altered epithelium and, during progression, epithelial changes occur at many points but not diffusely. The ample evidence of multifocal origin suggests that the whole epithelium is potentially malignant but, in general, progression from microscopic hyperplasia to gross papilloma and thence to carcinoma is essentially focal.[2,13,14,46,91]

Occupational tumors of the bladder, as found in dyeworkers, correspond in many respects with the experimentally induced tumors in animals. In conformity with the usual pattern of industrial cancer, the disease usually develops after a long period of exposure and often after a long period during which there is no further exposure to the presumed carcinogen. Goldblatt[73] records emergence of neoplasia at a mean period of about 19 years after first exposure and at varying times up to 12 years after leaving the industry. The tumors may be malignant at their first appearance, or they may present as benign tumors which either remain benign for a long time or undergo progression to carcinoma. In some patients, but not in all, there is a strong liability to the development of new tumors after destruction of the original one. Sometimes new tumors develop at or near the site of the old one but often they are distant from it, and new tumors can develop in various parts of the bladder throughout the patient's life.[72,73] The clinical observations indicate that exposure to the carcinogen brings about a widespread and permanent alteration in vesical epithelium; the alteration persists indefinitely after the withdrawal of the carcinogen and, although not visible, provides the basis upon which benign or malignant tumors can develop unpredictably and for no apparent reason at any time and at any place. Goldblatt thinks of the persistent change as a *potentiation* and that emergence of neoplasia may require the subsequent action of an endogenous carcinogen. In the light of more recent investigations of skin carcinogenesis in animals, it is reasonable to equate *potentiation* with *initiation* and to suppose that subsequent neoplasia may depend on unidentified promoting agents.

Familial Intestinal Polyposis.—In this familial disease, multiple tumors develop in childhood or early adult life as tiny nodules which later develop into small sessile growths or larger pedunculated polyps. The larger tumors have the histologic structure of adenoma. At first, all the growths appear to be nonmalignant, and most of them remain so but after a few months or a few years, one or more of them undergoes progression to carcinoma.[29] The disease resembles xeroderma pigmentosum in several respects; it has a hereditary basis, and progression to carcinoma occurs almost inevitably in every patient but in only one or a small proportion of the precursor lesions in each patient.

Neoplasia of the Uterine Cervix.—It is now widely accepted that precursor lesions recognizable by the examination of vaginal or cervical smears or of biopsy specimens are present in the cervix many years before the clinical emergence of invasive cancer. The lesion, known under various names, including carcinoma in situ, intraepithelial carcinoma, preinvasive carcinoma, and noninvasive carcinoma, is the best defined, and it is, in the general opinion, a frequent precursor

of invasive cancer. There is less agreement about the significance of a group of varied proliferative lesions described under a variety of terms including dysplasia, dyskaryosis, basal-cell hyperplasia, precancerous hyperplasia or metaplasia, atypical hyperplasia, and atypia. Reagan, Hicks, and Scott[133] list 15 of "the more commonly applied terms" of which "dysplasia" is chosen for the present discussion. The dysplasias include a wide range of epithelial proliferations which fail to meet all the criteria which most pathologists demand for a diagnosis of carcinoma in situ. It is admitted that there is no sharp division between dysplasia and carcinoma in situ; one merges into the other, and the differential diagnosis is often a matter of personal opinion; moreover the two kinds of lesions commonly exist side by side.[15,36,89,131,132,175]

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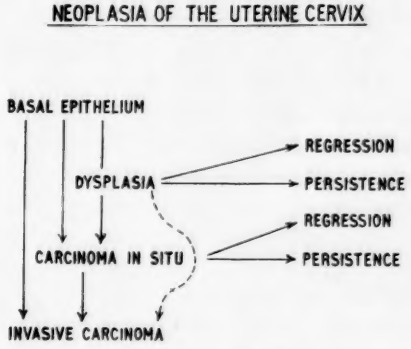

Fig. 1.—Paths of neoplasia in the uterine cervix.

The study of the natural history of cancer of the cervix received the greatest impetus from the introduction of the Papanicolaou smear and is pursued by the use of one or other of the modifications of that method as a "screening" test followed by biopsy for definitive diagnosis. It has been remarked that the serial biopsy method is best avoided, if possible, in tracing the natural history of tumors[50] and that it discloses, in fact, an "unnatural" history.[30] In the study of cancer of the cervix in women, the biopsy method is not currently avoidable; its limitations, which are adequately recognized, must be taken into account in the interpretation of observations.[89,133] There are three main pitfalls: (1) the biopsy specimen may give an incomplete and consequently misleading representation of the pathologic changes present in the cervix near to, or distant from, the site of biopsy; (2) biopsy may remove the whole of a precursor lesion so that no further development is possible; and (3) the biopsy, short of removing the whole lesion, may decisively change its subsequent course. In truth, the biopsy method rarely gives a direct demonstration of the provenance or fate of any particular lesion; some coexistent lesion not sampled at biopsy may be, with equal or greater probability, the starting point for subsequent development. A concept of the natural history of cancer of the cervix must be based on the cumulative evidence from pathologic, clinical, and epidemiologic investigations interpreted in the light of current knowledge of the general principles of neoplastic development. The scheme of development shown diagrammatically in Fig. 1

is not a representation of proved facts but a working hypothesis for further investigation, which, sooner or later, will confirm or refute its validity. The diagram shows dysplasia as a possible but not essential precursor of carcinoma in situ and the latter as a possible but not essential precursor of invasive carcinoma. There is substantial evidence that all the paths shown in Fig. 1 are, on occasion, followed; there is much less evidence to show how often.

Dysplasia: The incidence of dysplasia in the general population is not known, but it is admittedly much more frequent than carcinoma in situ and this, alone, implies that it does not inevitably progress to carcinoma in situ.[89,133] There is little doubt that the majority of dysplasias regress spontaneously, but some persist unaltered for long periods, some undergo progression to carcinoma in situ, and some, by-passing the in situ lesion, advance directly to invasive carcinoma.[63,65,89,130,132-134,175]

Dysplasia is present in 80 per cent or more of cervices containing carcinoma in situ.[15,131] Rawson and Knoblich[130] think that dysplasia is more commonly a warning sign of carcinoma in situ than a precursor of it. Reagan and Hamonic[131] record 9 cases in which carcinoma in situ developed in patients who had dysplasia on a previous examination; they believe that probably in 2 cases the carcinoma in situ developed from the dysplasia but that in the other 7 cases the two lesions were collateral and not consecutive. Galvin, Jones, and Te Linde[63] divide their 191 cases of dysplasia arbitrarily into three stages of development and find an increasing liability to subsequent development of carcinoma in situ with increasing severity of the dysplasia. They believe that although in the majority of cases dysplasia and carcinoma in situ possibly developed simultaneously, progression of dysplasia to carcinoma in situ probably took place. Dysplasia is found on the average about 6 years earlier than carcinoma in situ[133] which supports the opinion that they are consecutive processes.

Dysplasia, according to some observers,[36,119,133] is especially common during pregnancy and liable to regression after delivery, but others find no difference between the dysplasias in pregnant and in nonpregnant women.[130]

Carcinoma in situ: Carcinoma in situ probably develops from dysplasia as already indicated but also, on occasion, without this precursor stage.[134,175] There is evidence to show that it may suffer one of three fates.[104,127,134] It may undergo progression to carcinoma, it may persist for long periods without alteration, or it may regress. It is found, on the average, about 10 years earlier than invasive cancer and especially at the ages of 30 to 35 years.[129,175]

Invasive carcinoma has been found in patients in whom carcinoma in situ had been found at examinations made from a few months to 10 or 15 years previously.[15,63,65,129,132,134,175] Together these observations indicate a high probability that invasive carcinoma will develop in a patient in whom carcinoma in situ has been detected, although possibly not until many years later; with exceedingly rare exceptions they do not demonstrate that carcinoma in situ has undergone progression to invasive carcinoma, but there is sufficient collateral evidence to justify the provisional conclusion that this progression takes place. The age distributions of in situ and of invasive carcinomas run parallel but about 10 years apart; as the incidence of invasive carcinoma increases, carcinoma in

situ wanes. The differences in incidence among racial and ethnic groups are similar for in situ and for invasive cancer, both being less frequent in whites than in Negroes, and less frequent in Jews than in Gentiles. Histologic evidence often points to the supervention of invasiveness in a carcinoma in situ and, apart from invasiveness, carcinoma in situ and invasive carcinoma are histologically and cytologically similar.[84]

With more diligent search, the incidence of carcinoma in situ seems to be rising out of proportion to the incidence of invasive carcinoma.[101] The probable explanation is that some carcinomas in situ do not undergo progression to invasive carcinoma but persist without change for a long time or regress. Repeated examinations of patients yield evidence for long persistence and for regression.[104,127,134,143,175]

A disproportionately high incidence of carcinoma in situ has been reported in pregnant women, and many of the lesions regress after delivery.[36,119,129,175] In one series, however, 16 out of 19 carcinomas in situ, detected during pregnancy, persisted after delivery.[74,126] Some critics infer that the lesions that regress after delivery are not carcinomas in situ, but dysplasia. This seems to be a *post hoc* diagnosis based on the regression. Epperson and associates[36] state that at least five of the lesions in their series that regressed after delivery would have been diagnosed as carcinoma in situ if they had been found in nonpregnant women. Schleiffstein[144] describes "unusual lesions" of the cervix in 4 pregnant women; 2 of them regressed after delivery, and 2 persisted. Schleiffstein says that there are no absolute histologic criteria for differentiating these lesions. It is noteworthy that the tumors that regressed were in women aged 19 and 22 years and those that persisted were in women aged 30 and 32 years. The most reasonable interpretation seems to be that some carcinomas in situ are hormone-responsive and some are not and that with increasing age there is an increasing liability to progression from the responsive to the unresponsive state. As Galvin and Te Linde[65] maintain, carcinoma in situ should be considered strictly as a histologic diagnosis; this diagnosis should not be changed, although it properly may be qualified, to meet observations outside the field of histology.

According to current opinion, many and probably the majority of invasive cancers of the cervix develop in women who have had carcinoma in situ for periods up to 10 or 15 years. It is likely, but not proved, that most of these cancers develop by progression in a carcinoma in situ but that some develop more directly by progression from dysplasia or from apparently "normal" epithelium. It is not known what proportion of dysplasias progress to carcinoma in situ or what proportion of carcinomas in situ progress to invasive carcinoma, nor is it possible to determine in advance which of the lesions will undergo progression or when. There is adequate evidence to show that the lesions can persist unchanged for long periods and that dysplasia regresses frequently and carcinoma in situ less frequently. There is a possibility deserving more attention that there is a progression in carcinoma in situ and possibly in dysplasia from hormone-responsiveness to unresponsiveness.

The inescapable difficulties in the analysis of the natural history of cancer of the cervix are aggravated by an inadequate nomenclature; knowledge of

neoplastic development has outrun the vocabulary for describing it. It is arguable whether or not the term carcinoma, however qualified, should be used for precursors of invasive carcinoma; it cannot be so used if the word carcinoma is used to imply an inevitably progressive and fatal disease.[87,110] There being no evident alternative, it seems best for the time being to retain the word carcinoma but to use it in a limited and clearly defined sense. Every carcinoma is at the beginning in situ, preinvasive, and intraepithelial, at least transitorily, until it has invaded. Carcinoma in situ of the cervix is not a mere transitory stage in a continuous extension in space and time and should be distinguished therefrom as a clinical entity and an enduring stage in a discontinuous process of development. Carcinoma in situ differs from invasive cancer qualitatively and not merely temporally. According to current views of neoplastic development, carcinoma in situ is noninvasive because it lacks the power of invasion. It is more properly noninvasive as so described by Galvin and Te Linde[65] than preinvasive. The latter term is misleading; the in situ lesion does not inevitably become invasive, and it never does so until it has undergone irreversible qualitative change or progression whereby it acquires a new capacity and becomes a neoplasm qualitatively different from what it was before. Carcinoma in situ and invasive carcinoma differ by the unit character of invasiveness. Whether or not carcinoma in situ regresses is a question for observation and not for argument; the present indications are that it does. Carcinomas in situ may differ among themselves in hormone responsiveness; moreover this unit character in accordance with the principle of independent progression of characters may combine with the morphologic characteristics of either dysplasia or carcinoma in situ.

Carcinoma in Situ.—Fennell and Castleman[39] review a wide variety of lesions in many different sites, to which the term carcinoma in situ has been applied. It is not clear that all the lesions deserve the name which is a useful one only if it is used for a localized lesion that differs qualitatively from invasive carcinoma and persists with that qualitative difference for a substantial time. Bowen's disease and erythroplasia are reasonably included, and acceptable examples have been recorded in cornea and conjunctiva, larynx, lung, and gastrointestinal tract. A preinvasive stage of adenocarcinoma of the endometrium and endocervix seems to occur frequently but is more difficult to detect during life than carcinoma in situ of the cervix.[3,59,82,83,156,157]

Franks and Chesterman[57] describe intraepithelial carcinoma of the prostatic urethra, periurethral glands, and prostatic ducts. Some intralobular growths of the breast are of the in situ type but the term is less justified for some tumors, notably intraduct carcinoma, where the tumor is not localized or intraepithelial, although it probably lacks invasive powers.

Neoplasia in the Breast.—The study of the natural history of neoplasia in the human breast has centered mainly on the attempted identification of "precancerous" lesions and, most controversially, on the significance of "chronic mastitis." Chronic mastitis is an objectionable name, because it implies that the disease is inflammatory, which it is not. The alternative term "fibroadenomatosis" avoids this implication and indicates the association, in varied degrees, of epithelial and connective tissue proliferations, but the close resemblance of

the word to "fibroadenoma" is unfortunate. The terms have clinical value but no precise pathologic meaning. They cover a variety of named lesions including mazoplasia, cystic hyperplasia, papillomatosis, blunt duct adenosis, and fibrosing adenosis. Many of these lesions are probably etiologically related, and some are local variations or different stages of a single process; the separation into named types is more a matter of convenience than of principle, and the convenience is minimized by the plethora of synonyms.

Mammary neoplasia advances along two main paths. One path, for which the term *centrifugal* is proposed, entails growth in a predominantly centrifugal direction from the mammary tree and differentiation toward myoepithelium and connective tissue; it leads to the variety of chronic mastitis called mazoplasia, to fibrosing adenosis, and to fibroadenoma with its varied modifications and derivatives including sarcoma and "mixed" tumor. The other, *centripetal*, path

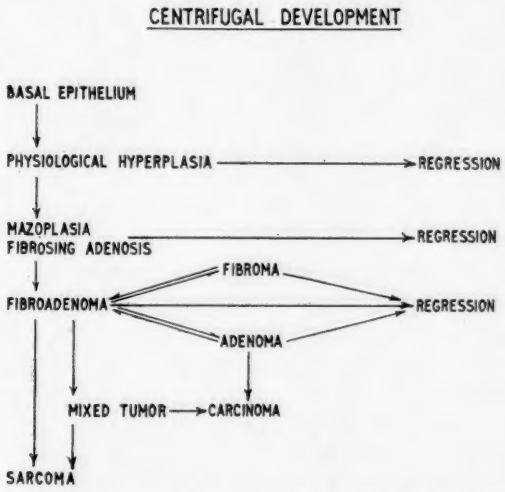

Fig. 2.—Centrifugal paths of mammary neoplasia.

involves growth that is primarily centripetal in direction and differentiation toward the epithelial type; it leads to cystic hyperplasia, papillomatosis, and carcinoma. Both pathways probably start from the same point, namely basal mammary epithelium, but this controversial opinion is not essential to the present discussion which is restricted to consideration of progression along each of the pathways separately and not to their mutual relationship. The division into two pathways is provisional; it is probably too crude and diagrammatic but serves at least to clarify some of the complex problems of mammary neoplasia.

The centrifugal path: The proposed paths of development are shown diagrammatically in Fig. 2. There are three possible consequences of physiologic hyperplasia namely (1) reversion to normal epithelium, (2) regression, and (3) progression to mazoplasia or fibrosing adenosis. By contrast with reversion, which implies a return to the original state, regression and progression are irre-

versible processes leading, the one to elimination of the hyperplastic tissue and the other to a new kind of tissue.

Cheatle and Cutler[18] describe mazoplasia as a hyperplasia of epithelial cells in ducts and acini, accompanied by the formation of new ducts and acini. A similar condition is present normally at birth, disappears during infancy to reappear in girls at puberty and thereafter subsides until evoked again by pregnancy. Minor degrees of mazoplasia are almost "normal" in women who have borne children, but the changes range from this minimal abnormality to a painful disease with palpable irregularities or lumps in the breast. Mazoplasia may be attributable, as Cheatle and Cutler believe, to a localized persistence of physiologic hyperplasia but it is not, on that account, a "normal" or "physiologic" condition; the localization and the persistence are abnormal and pathologic and are evidence of an irreversible change in reactivity and behavior. The severely painful mazoplasia is certainly pathologic and more than a persistence of a physiologic change. Mazoplasia seems to be hormone-responsive as indicated by the aggravation of symptoms for a few days before and during menstruation and by its regression at the time of the menopause. The alternative fate of mazoplasia is progression to fibroadenoma.[18]

Similar considerations apply to fibrosing adenosis, encountered most frequently in young women. The lesions may regress in time or undergo progression to fibroadenoma. Fibrosing adenosis further resembles mazoplasia in being similarly responsive to the menstrual cycle and in its trend toward progressive fibrosis whose myoepithelial element is especially evident.[26,79,96,164]

Progression in mazoplasia or less often in fibrosing adenosis leads to fibroadenoma. Despite all histologic resemblances to normal mammary lobules and all arguments based thereon, fibroadenoma is abnormal, pathologic, and neoplastic. Fibroadenoma is found most commonly in young women. It reacts to the menstrual cycle like normal breast tissue,[95] and it is variously responsive to pregnancy and lactation, some tumors responding by epithelial proliferation and secretion, some by proliferation without secretion, and some not at all.[68]

Fibroadenoma in rats is transplantable and both primary and transplanted tumors are notably hormone-responsive.[124] When stimulated by pregnancy or the administration of estrogens, fibroadenoma undergoes reversible modulation to adenoma or secreting adenoma whereas, under androgenic stimulation, the epithelial component dwindles and the tumor becomes a fibroma. Under undefined conditions, squamous epithelium and cartilaginous or bony tissues develop in fibroadenoma. Reversion to fibroadenoma, as a result of further transplantation, shows that these changes are reversible modulations to be distinguished sharply from irreversible progression. Progression to sarcoma occurs rarely in primary tumors but often in transplanted ones. Progression to carcinoma is rare. Fibroadenoma is common in dogs and closely related to the well-known "mixed" mammary tumors which comprise carcinomatous tissue, often of squamous epithelial type, sarcomatous tissue and conspicuous admixtures of cartilage and bone.

The observations on fibroadenoma in animals help in the interpretation of some uncommon processes in women. Pure adenoma, rare in women, is attrib-

utable to reversible modulation in a fibroadenoma whose epithelial component has become predominant and functional in response to hormonal stimulation, probably estrogenic. Secretion in the tumor parallels that in normal breast and, during pregnancy and lactation, leads to *lactating adenoma.*[18,25,85,122] Exceptionally, the modulation may become fixed as a progression, and an adenoma gives origin to a carcinoma.[85] Much more commonly, as in rats, progression is toward sarcoma or "mixed" tumor. Progression in fibroadenoma leads to *giant fibroadenoma,* benign or malignant *cystosarcoma phyllodes,* or *sarcoma.* The progression is sometimes recognizable histologically as a focal alteration within the original tumor.[18,106,158,161,172] Fibroadenoma and cystosarcoma sometimes contain squamous epithelium,[123,161,172] and cystosarcoma is sometimes still more complex including areas resembling squamous carcinoma and osteogenic sarcoma with atypical cartilage and bone.[18,172] A complex tumor described by Tudhope,[163] believed to have originated in a fibroadenoma, comprised carcinomatous and fibroadenomatous tissues as well as pseudocartilage, true cartilage, and bone. It links fibroadenoma with the rare mammary tumors described by Stewart[158] as "mammary carcinoma with osseous and cartilaginous metaplasia." Stewart states that not a few of these tumors contain areas like squamous carcinoma, and he recognizes their similarity to the "mixed" mammary tumors of dogs.

Centrifugal development does not advance automatically to its possible termination in sarcoma or carcinoma, and most frequently it does not so advance within the lifetime of the patient. The process can stop for a long time or permanently at any step in the sequence; reversion probably cannot occur after the first step, but there is a choice between persistence, progression, and regression at every step except the last. Development does not necessarily traverse all the known steps in sequence; it may vault or by-pass one or more of them. Although information is scanty, it is likely that fibroadenoma may develop without preceding mazoplasia and sarcoma without preceding fibroadenoma. Many of the more complex tumors probably develop by modulation and progression in fibroadenoma, but others may follow a peculiar by-path of their own from the beginning and avoid the stage of fibroadenoma or pass through it rapidly and inconspicuously. These alternative paths are omitted from Fig. 2, which is almost certainly oversimplified.

The centripetal path: Fig. 3 shows some of the probable paths of centripetal development. It shows on the left a *direct* path to carcinoma which traverses no recognizable intermediate stages or precursor lesions. Although proof is hard to find, there is evidence for this direct path in mice[50,66] and in women.[117,172] The other paths are indirect, traversing intermediate stages. Most carcinomas of the human breast originate in ducts, but intra-acinar carcinoma also occurs in conjunction with intraduct carcinoma or as the sole or predominant tumor.[18,42,117] Hyperplasia, shown as an intermediate stage in the diagram, may occur diffusely and simultaneously in a large number of acini, and the diffuse hyperplasia may advance to carcinoma. There is a noninvasive stage of carcinoma in situ which is not often recognized; this may change into an invasive carcinoma, which is highly malignant.[42,43,117,158] Judging from one report, the in situ stage may last for 12 years before it undergoes progression to carcinoma.[71] The carcinomatous

hyperplasia, recently described by Jackson and Orr,[99] is a possible precursor of cancer whose importance cannot yet be assessed. The most controversial indirect path is that through cystic hyperplasia, and it illustrates the avoidable confusion about precancerous lesions that hitherto has obstructed the study of the natural history of neoplasia.

The indirect path through cystic hyperplasia and papilloma to carcinoma, as illustrated in Fig. 3, corresponds with the account by Cheatle and Cutler.[18] Cheatle and Cutler describe a disease that may advance through three distinct stages. The first stage of *cystic hyperplasia* (called by them *cystiphorous desquamative epithelial hyperplasia*) is encountered most frequently in women about 25 to 35 years old. It begins with desquamative epithelial hyperplasia in terminal ducts and acini. Cysts develop as the result of the secretory activity of epithelial cells; they increase in size and fuse together to make larger cysts.

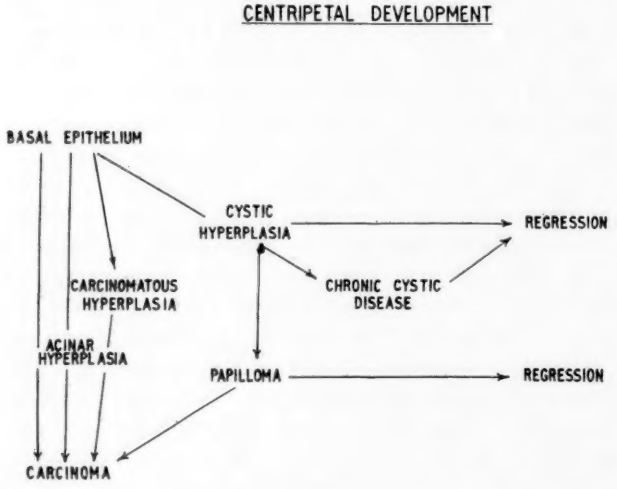

Fig. 3.—Centripetal paths of mammary neoplasia.

The disease may halt for a long time or permanently at this cystic stage or it may advance to the next stage of *benign neoplasia* which is found usually about a decade later than cystic hyperplasia in women about 35 to 45 years old. Usually only a fraction of the hyperplastic epithelium is affected, but sometimes nearly all of it changes. There are two main forms of benign neoplasia, namely papillomas with connective tissue cores and sessile epithelial proliferations devoid of stroma. The benign hyperplasia may persist for a long time or permanently, or it may undergo progression to the third stage of *malignant neoplasia* which is found, about a decade later than benign neoplasia in women aged 45 to 55 years. Judging from histologic appearances, malignant neoplasia develops from benign neoplasia and not directly from cystic hyperplasia. Progression is usually restricted to a small area or multiple small areas of the benign neoplasia, but sometimes it extends over large areas. All three stages and transitions from

one to the other may be present in one breast at the same time and the complex mixture of hyperplastic and neoplastic lesions constitutes Schimmelbusch's disease.

Cheatle and Cutler emphasize some important features of this sequence which later writers have insufficiently stressed. In particular they insist that the three stages represent three distinct processes biologically and morphologically different from one another and maintain that the disease may halt for a long time or permanently at the first or second stage. They recognize, in other words, that the advance is discontinuous, that progression from one stage to the next involves a qualitative change and not a mere intensification of a process already in being and, further, that progression does not advance automatically or inevitably through all stages of the sequence.

The diagram (Fig. 3) shows a side-branch of development from cystic hyperplasia to "chronic cystic disease" by which is implied the clinically evident "chronic mastitis" with cysts. This development from cystic hyperplasia to cystic disease may involve progression because it seems to entail the loss of the capacity for further progression along the neoplastic pathway; it is widely agreed that carcinoma rarely develops from fully-developed cystic disease.[4,12,18,43,125] On the other hand, there is statistical evidence to show that women with a history of cystic disease are four or five times as prone as other women to develop cancer of the breast. It is remarkable that the cancer develops as often in the breast in which no cystic disease had been found as in the breast where it was present.[168] The probable interpretation of these observations is that clinical cystic disease, although no longer capable of progression to neoplasia, is an indication that cystic hyperplasia, a potential precursor of cancer, has been present in the same breast, and possibly in the other breast as well, and that both breasts, have been exposed to at least one of the etiologic factors that encourage mammary neoplasia.

Summarizing the preceding argument, cystic hyperplasia is liable to progression to papilloma and thence to carcinoma, but it is also liable to progression to chronic cystic disease which is not capable of progression to neoplasia. Papilloma is liable to regression as well as to progression to carcinoma.[18,43] The steps in the sequence do not follow one upon the other automatically or inevitably. Reversion is not possible after the first step in the sequence, and not certainly then, but regression is possible at all stages up to carcinoma and further progression is possible at all stages, even in carcinoma as shown by innumerable transplantation experiments in animals. It is probable that steps in the sequence can be by-passed and that papilloma, for example, may develop without preceding cystic hyperplasia.[18,43]

The study of paths of progression can go far to reduce the intolerable and sterile controversies about "precancerous" lesions, but many questions of fact remain unanswered. It is not known how many paths lead to carcinoma, nor how often any of them is followed. Cheatle and Cutler thought that 25 per cent of their cases developed through cystic hyperplasia whereas the other 75 per cent probably did not. Willis[172] thinks that 25 per cent is an underestimate, but no approach to an agreement seems imminent. The percentage of all women who

have cystic hyperplasia and the percentage of women with cystic hyperplasia who develop carcinoma are unknown. Other paths of development certainly exist and deserve closer attention. One of the purposes of Fig. 3 is to illustrate graphically the multiplicity of paths of development.

A general feature of mammary tumor development along indirect paths is the progressive restriction of fields of progression. In mice the whole mammary tissue is prone to neoplasia, but only some portions of it advance to the first visible stage of nodular hyperplasia or plaque formation. Of the nodules, only a few undergo progression to carcinoma, and in the plaques, as a rule, only a portion of the available tissue undergoes that progression. In gross tumors, progression is sometimes demonstrable histologically, and it involves only a small fraction of the whole tumor. In mice, progression is most often focal or multifocal, but it may be regional, diffuse, or even total.[50] In neoplasia of the human breast, progression conforms to the same pattern; it is often focal or multifocal or restricted to small portions of the field of potential development. Less commonly, progression is more diffuse or regional rather than focal, and much more rarely it seems to be total or subtotal.

Progression may, but does not inevitably, occur in some portions of a widespread area of tissue predisposed to neoplasia to yield regions of altered reactivity which may or may not be visible as "lesions." One or more steps of this kind may precede the initiation of a process that is recognizably neoplastic; even then neoplasia may advance through stages, at each of which there is a choice between a further advance, a temporary or permanent halt or regression, before it reaches a stage of inevitably progressive growth. On some pathways there are two or three potential precursors of carcinoma; each may undergo progression to carcinoma but none does so inevitably. Lesions of similar kinds may be present in breasts containing cancer and in breasts free from cancer; they may yet be potential precursors of cancer whose further development depends upon some additional etiologic factor not essential for their own origin or persistence. The extrinsic or intrinsic factors upon which progression depends are unknown but in the consideration of etiologic factors, it is clearly necessary to bear in mind the possibility of different factors acting at different stages of progression. Judging from laboratory as well as clinical observations, the initiation of mammary neoplasia in women is likely to precede the clinical detection of cancer by two or three decades.

THE COURSE OF NEOPLASIA

Clinical Course.—The clinical course of cancer is not always steadily and relentlessly progressive. Remissions and exacerbations occur unpredictably and not infrequently.[27,28,32,33,94,153] Survival statistics yield average figures which have little relevance in clinical practice where each case differs to a greater or less extent from all others.[111] Untreated cancers kill 75 per cent of the patients within average periods ranging from 14 months for cancer of the esophagus to 46 months for cancer of the breast, but long survivals are possible even with those types of cancer that, as a rule, are rapidly fatal.[147] Sometimes the course is surprisingly prolonged. In a study of untreated breast cancer, Daland[24] found that, although the majority of patients died within 3 years, 22 per cent lived for

5 years and 5 per cent for 10 years. The length of survival was not evidently correlated with the clinical estimate of "malignancy." Nathanson and Welch,[118] continuing Daland's investigation, record one survival for 15 years. Survivals after incomplete eradication of cancer are similarly varied and unpredictable. Not uncommonly patients survive for 10 or 15 years before dying of recurrent or metastatic growth and the long survivals are not correlated with any evident peculiarity of the tumors.[103,116,147,171]

The results of treatment are unpredictable in individual patients; there are unaccountable failures as well as unexpected successes. There are "good" and "bad" cancers[28]; the "good" cancers are curable by almost any reputable treatment, whereas the "bad" ones respond to none. Macdonald[108] and Denoix[28] deny that "curable" cancer differs from the "incurable" only in being "earlier" or less advanced; they maintain that the two kinds of cancer are not different stages in one process but distinct processes different from the beginning. In his hypothesis of "biological predeterminism," Macdonald maintains that the various forms of cancer in one organ have different natural histories. He believes that the "biological potential" is determined early in the preclinical phase and that the inherent "growth potential" of the tumor and whatever balancing defensive mechanisms it may excite in the host together constitute a biologic complex that determines the course of the disease. At the time of early clinical detection the "growth pattern" is already established and the clinician has to deal with a disease of biologically determined potentialities; rigid ideas of prognosis based upon duration and size should be abandoned in favor of the attempt to evaluate the "biological potential" of the tumor in its individual host.

The important and valid implications of Macdonald's hypothesis are that each tumor has its own individuality determined by innate characters of the tumor cells and that the individuality is often determined early in the course of neoplasia. The word predeterminism is unfortunate if understood to mean that the definitive characters of a tumor are always and irrevocably fixed at an early stage in its development. Moreover, the hypothesis is weak and evasive in attributing the individuality of tumors to their "biological potential," an abstraction like "essential malignancy," "growth potential," "growth pattern," and similar terms used in a like sense. These abstract ideas need translating into concrete terms by resolving the behavior of tumors into the several characters that contribute to it.

Denoix[28] has proposed a provisional classification, which, although not wholly acceptable, gives a more practical basis for investigation and discussion. Denoix divides cancers into four main types, which differ from the outset: (1) The *localized* type is limited to the organ of origin; it is not merely an "early stage" in the development of other types but a particular type without tendency to generalization. (2) The *regional* type exceeds the limits of the organ by direct extension without spreading to the lymph nodes. (3) Tumors of the *lymphophil* type have already invaded the regional lymph nodes when the primary tumor is first recognized clinically. (4) The *diffused* type is remarkable because, at the time of clinical recognition, the whole body is seeded with tumor cells whose evolutionary potential is revealed at unpredictable times thereafter.

Denoix and others[27,94,108,170] discard the orthodox view that neoplasia advances through an orderly sequence from local invasion to progressive lymph node invasion and thence to blood-borne metastasis and propose, instead, that in many patients there is an early and simultaneous widespread dissemination with subsequent wide differences in the fates of the disseminated cells. Denoix believes that tumors usually disseminate before the first clinical symptoms appear and that the number of secondary deposits that advance to clinical recognition bears no relation to the number of metastatic deposits of microscopic size. The disseminated cells are subject to three fates: (1) they may die; (2) some may die and the others survive in a dormant state whence they emerge unaccountably, at unpredictable times, and grow into secondary tumors; (3) they may multiply promptly to form secondary tumors.

Denoix adduces evidence to show that growth rate, local invasion, spread to regional lymph nodes, and dissemination by the blood stream are independently variable characteristics. His evidence supports the proposition that the behavior of tumors is determined by unit characters that are independently variable and liable to combination in a variety of ways. The "essential malignancy," "biological potential," or "growth pattern" of a tumor depends upon a particular association of unit characters. The possible combinations of unit characters are extremely numerous so that each tumor is a particular entity differing from all others.[27,111]

Metastasis is a complex process; several steps must occur in sequence before remote secondary tumors can become clinically evident.[51] The essential steps are as follows: (1) *liberation* of tumor cells from the primary growth; (2) *transportation* of the liberated cells away from the primary site; (3) *deposition* of the transported cells in a distant site; (4) *establishment* of the deposited cells, by which is meant the establishment of vascular and connective tissue unions with the surrounding tissue; and (5) *growth* of the established deposit into a secondary tumor. Many factors operating at different stages of the sequence can decide the final result. The availability of transport in the form of an abundance of vascular or lymphatic channels probably accounts for the high probability of metastasis from tumors in certain situations.[38] Several factors, including inappropriate surgery and trauma from excessive clinical manipulation, probably favor liberation.[28,153] Trauma may also favor deposition. The latter steps are poorly understood. It is certain that many of the transported cells fail to originate secondary tumors and that many of them die.[44,145,171] Removal of a primary tumor possibly discourages the growth of metastatic deposits or even leads to regression of secondary tumors; at least, it prevents further seeding, and it is possible that small numbers of cells can be held in check whereas large numbers cannot.[28]

French writers emphasize the irregularities in the clinical course of tumors and in particular the "poussées évolutives" which occur unpredictably in many patients. Some of these outbursts seem attributable to progression to a new and more aggressive type of neoplasia, but others are not so readily explicable and the scarcely explored possibility of alterations in body "resistance" cannot be ignored.[27,28,93,94]

Anomalies of Behavior.—Many anomalies in the behavior of tumors are attributable to unexpected, but not necessarily uncommon, associations and dissociations of unit characters. Disproportionate or "out-of-step" progression of characters is exemplified in the "locally malignant" tumors that invade vigorously but do not metastasize and the "metastasizing benign tumors" that disseminate without conspicuous local invasion.[47] Basal-cell carcinomas sometimes metastasize and the "metastasizing benign tumors" are, in truth, invasive but the disproportion between local invasiveness and dissemination is extreme. The following paragraphs discuss some of the more conspicuous anomalies in the behavior of tumors.

Occult primary tumors: Occult primary tumors, closely allied to the "metastasizing benign tumors" are undetected primary tumors that have given origin to clinically manifest secondary growths. The precocious dissemination presumed by Denoix is here apparent. Secondary tumors are apt to be the presenting sign, drawing attention to occult primary tumors in certain sites, notably thyroid and lung.[171] Occult primary tumors of the breast give origin to gross secondary tumors in the axillary lymph nodes or, less commonly, in bones, causing spontaneous fracture, before the primary tumor is clinically detectable even if it is suspected and specifically sought.[18,20,28,41,97,171] The mechanism of the precocious dissemination is unknown; it deserves the closest investigation as the most serious bar to satisfactory control of some cancers, especially of the breast.

Dormant tumors: The concept of dormancy is evoked to account for the long-delayed recurrences and metastatic growths after apparently complete removal or destruction of the primary growth. Delays of 10 or 15 years are not uncommon and sometimes, it is said, they extend to 40 or 50 years.[77,171] As judged by the ultimate distribution of secondary growths, the early dissemination, in accordance with Denoix's belief, is extremely widespread. The reasons for the dormancy and for the ultimate emergence from the dormant state are obscure. Hadfield[77] believes that the tumor cells survive in mitotic arrest in an altered state which enables them to withstand separation from their parent growth until they acquire a new blood supply. Shimkin and associates[148] argue that if the tumor emerges as a consequence of progressive growth throughout the period of dormancy there should be a relationship between the duration of the dormant period and the length of life after emergence whereas, if host factors decide the emergence, the length of life thereafter should be independent of the period of dormancy. Their own investigations give no decisive support to either alternative; the earlier observations of Truscott,[162] showing that emergent mammary tumors grow with equal rapidity, irrespective of the length of the dormant period, are consistent with the second.

Steady progressive growth, however slow, seems an unlikely explanation for the emergence from dormancy lasting for up to 40 to 50 years. According to Dunphy[33] the secondary tumors do not advance simultaneously or in parallel, which implies that local factors predominate over systemic ones. Unidentified extrinsic factors may "trigger" the emergence, or the emergence may be due to progression, which, as experimental investigations show, can occur in dormant tumors. Dormancy is commonplace in transplanted tumors but no better under-

stood except in the special case of hormone-dependent tumors which may be dormant for long periods to emerge only when the required hormonal stimulus is supplied.

Latent carcinoma: The original observations of Rich[135] on the frequency of clinically unsuspected cancers found post mortem in the prostates of men past middle age have been amply confirmed.[34,54,55,56,86] Although the designation "carcinoma" is agreed, the relationship between the latent tumors and the clinically evident ones is not settled. Franks[56] distinguishes between *latent* or *unsuspected* cancers and *active* or *clinical* cancers. The latent tumors are found especially in old men; they are increasingly common after the age of 80 and are present in most men over 90. They seem to grow very slowly. They may be large or small. Histologically they may be well differentiated or anaplastic and are indistinguishable from clinical cancers; they commonly invade blood vessels and lymphatics but distant secondary tumors rarely develop. The difference between latent and clinical cancer is a biologic one recognizable only by the behavior of the tumor in its host. The division is not a sharp one and "latency" probably exists in varied degrees. It is estimated that about 9 per cent of patients with clinically diagnosed cancer of the prostate survive for 5 years or longer without treatment. Apparently, tumors can grow to clinically recognizable size and yet retain the biologic characteristics of latent tumors.

On balance, the available evidence suggests that latent and clinical cancers are qualitatively different from the beginning and not merely consecutive stages of a continuous process of growth and extension. They differ in certain unit characters that are expressed in growth rate and, more remarkably, in the failure of the latent type to yield secondary tumors despite local invasiveness. Latency may depend on the lack of unit characters needed for the completion of all the steps in metastasis or, as Franks[56] suggests, it may have a hormonal basis or be associated in an unexplained way with aging. Some clinical cancers develop from latent ones, but all latent cancers certainly do not develop into clinical cancers. Latent cancers change into clinical cancers, it seems, not through mere extension, but as a result of qualitative progression.

In a series of 1,300 autopsies on patients over 70 years old, McKeown[113] found 261 cases of malignant disease of which 20 per cent were "latent" in the sense of being unsuspected during life and not materially responsible for death. The latent tumors included carcinomas of the gastrointestinal tract and lung as well as carcinoma of the prostate. Others have reported latent carcinoma in kidney, thyroid, breast, and lung.[55]

It seems desirable to exclude "occult" primary tumors and carcinoma in situ and, taking the latent tumors of the prostate as the prototype, to restrict the term *latent carcinoma* to histologically invasive, carcinomatous tumors that do not metastasize and that are persistently and qualitatively different from clinical cancer and not merely transitory stages in its development. Latent carcinomas may exceed the threshold of clinical recognition without losing these characteristics, but to become typical clinical cancers they must undergo qualitative progression. Latent carcinoma differs from occult cancers in the absence

of metastasis; it resembles carcinoma in situ in clinical latency but differs from it in at least one unit character, namely, invasiveness.

Hormone dependency: The current theory upon which endocrine therapy of cancers of the prostate and breast is based presumes that these tumors are hormone dependent and grow only when they are stimulated by appropriate hormones. Huggins[92] writes: ". . . When the original cells are dependent upon hormonal support for metabolic activity at a high rate, its cancers can be similarly dependent and these atrophy when hormonal support is withdrawn by any of a number of means; these cancers are by definition dependent tumors. . . ."

Hormone-dependent growth is an example of *conditional* growth. It is not an all-or-none phenomenon; there are varied degrees of hormone responsiveness between the extremes of hormone-dependence and hormone-independence. Moreover hormone responsiveness is manifested in other ways besides growth, and tumors show a wide range of qualitatively as well as quantitatively different responses to hormones.[49]

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The hormone responses and hormone dependence of tumors have been discussed in several reviews.[10,48,49,62,124] It is noteworthy that, whereas much of the experimental work has dealt with hormone-induced tumors that are dependent for growth as transplantable tumors upon an abnormal endocrine environment, the present indications are that cancers of the human breast and prostate grow in an endocrine environment that is apparently "normal" and are checked when the normal balance is disturbed. The hormone decisively concerned in maintaining neoplastic growth is sometimes but not necessarily one prominent in inducing that growth originally.[165]

The various forms of hormone responsiveness are unit characters independent within wide limits of other characters and liable to independent progression. Consequently, hormone dependence or hormone responsiveness is not well correlated with other clinical or pathologic features of neoplasia. Cancer of the prostate, as an example, may show all the orthodox criteria of malignant growth and yet be strongly responsive to hormonal control. The persistence of hormone responsiveness at late stages of malignant neoplasia is one of the most unexpected recent discoveries of cancer research. Within limits the hormonal stimulus is amenable to experimental or therapeutic control, but the responsiveness of the tumor is an intrinsic character, not predictable from its structure or behavior, which may change by irreversible progression so that the same stimulus produces a different effect or none at all.

Regression: Complete regression of malignant neoplasia is undoubtedly rare, but examples satisfying reasonable criteria of observation and diagnosis are on record.[10,32,33,37,147,159] Everson and Cole,[37] reviewing 600 reputed examples, accept 47 as authentic; their accepted cases include neuroblastoma (10), cancer of the bladder (8), malignant melanoma (5), cancer of the breast and soft tissue sarcoma (4 each), and 1 or 2 cases of each of a wide variety of malignant tumors.

Neuroblastoma, highest in the list of regressing tumors, is liable to change into the less aggressive ganglioneuroma by a process that seems akin to maturation. More frequently, ganglioneuroma undergoes progression to neuroblastoma, but it is of considerable interest that the reverse process occurs at all.

Spontaneous arrest of growth and retrogressive changes occur much more frequently in benign than in malignant tumors; they are not infrequent in adenomas of the thyroid or breast, in uterine myomas, and in lipomas, fibromas, and meningiomas.[172] Bielschowsky[10] draws attention to the remarkable but apparently waning disease, papilloma of the larynx in children. The papillomas are multiple and may be very numerous and extend into the trachea. As a rule they disappear spontaneously about the time of puberty but may persist into early adult life. There is a record of disappearance of papillomas in a young woman during each of three pregnancies and reappearance of them with the re-establishment of menstruation after delivery.[88] There is evidence therefore of an unexpected hormone responsiveness whose mechanism has not been elucidated. Although the majority of tumors regress at puberty or in early adult life, progression to carcinoma occurs in a few patients and in papillomas which have been present for 4 to 30 years.[16,98,121,167]

Bielschowsky[10] gives a valuable review of regression of hormone-dependent tumors following hormone withdrawal or disturbance. Some believe that the primary action of endocrine therapy is to stimulate a defensive reaction of connective tissues[35]; others give evidence for a primary degeneration of the epithelial parenchyma.[40,53] During regression of responsive mammary tumors in mice, epithelial degeneration and connective tissue changes seem to advance concurrently; epithelial degeneration possibly starts first and is far advanced before connective tissue changes are sufficiently great plausibly to account for the regression.[50]

Conclusions

The abundancy and reliability of the information about the natural history of neoplasia depends to a great extent on the accessibility of the primary site to direct observation. Nevertheless, a survey of varied types of neoplasia reveals a pattern of development common to all of them. A recurrent feature, inconsistent with the widely accepted view of the inevitable and implaceable advance of neoplasia along a predetermined course, is the frequency of a choice of pathway. Neoplasia can advance in many different ways to similar or diverse fates. There are several paths to carcinoma or sarcoma, and at many stages of development a diversion of the path toward other destinations is possible.

In many forms of neoplasia there is evidence of a wide field of "prepared" tissue within which neoplasia develops focally. Laboratory investigations show that widespread "initiation" can occur without gross or histologic change and that the added stimulus of "promotion" evokes visible focal lesions of varied kinds. Two main pathways are distinguishable from the outset, namely a *direct* path whereby carcinoma develops from the initiated tissue as the first visible lesion and an *indirect* path which traverses one or more stages, not necessarily recognizable as neoplastic, before it leads to carcinoma. Stages on the indirect path are described variously as precancerous lesions, precanceroses, preneoplastic lesions, precursor lesions, or benign tumors. All the terms are, to a greater or less extent, objectionable or misleading. At each "precancerous" stage there is a choice of path leading to at least three different fates: (1) extinction or con-

version into a chronic disease without neoplastic qualities as in chronic cystic disease of the breast; (2) persistence, and perhaps growth, without qualitative change; and (3) progression toward malignant neoplasia involving irreversible qualitative change in one or more characters and conferring new properties. The lesions along the indirect paths are *potential precursors* of cancer; they are not predestined to develop automatically into cancer, and as a matter of observation this is their least likely fate; they are much more likely to persist as they are, or regress. The probability of progression to carcinoma varies greatly in different forms of neoplasia, but even when there is an overwhelming probability that cancer will develop, as in xeroderma pigmentosum or familial intestinal polyposis, only a proportion of the "precursor" lesions undergo progression to carcinoma. Although the majority of malignant melanomas develop by progression from nevi, the percentage of all nevi that undergo progression is extremely small. The significance of "precancerous" lesions is two fold: (1) one or more of them may, with varied probability, undergo progression to malignant neoplasia, but none does so inevitably; (2) the lesions provide evidence that a carcinogenic stimulus has been effective and that "initiation" of neoplasia has occurred so that carcinoma may develop from other areas where no "precancerous" lesions have been present at any time. It seems to be a general rule that however frequently cancer originates from precursor lesions it can also spring, by *direct* development, from "initiated" but not visibly altered tissue. In some forms of neoplasia *direct* development may predominate over the *indirect*. Moreover, as shown certainly in the skin, and with high probability in the breast, there are several indirect paths to carcinoma, and on each of them there are repeated choices between regression, persistence, and progression toward carcinoma. An important feature of all precursor lesions is that their progression to carcinoma is not inevitable; they are all neoplastic but "precancerous" only in the sense of a greater or less degree of probability of progression to cancer. The probability of progression in carcinoma in situ seems to be high, but it is not proved that all the invasive cancers develop from in situ lesions and not from other "initiated," but not obviously abnormal, areas of the cervix.

The difficulties in naming the lesions on the indirect paths and many definitive tumors, however evolved, are attributable to the greatly varied associations of unit characters for which the orthodox pathologic classifications of tumors make no provisions. The lesions on the indirect paths are neoplastic, but they are not necessarily recognizable as "benign tumors" or "malignant tumors" the distinction between which is at best arbitrary[172] and based on the clinical behavior of advanced stages of neoplasia. The precanceroses, the dysplasias, the carcinomas in situ, the latent tumors, the occult tumors, and the hormone-dependent cancers are alike in possessing particular associations of unit characters for which none of the orthodox terms is appropriate. A new language is needed to express the new outlook on the development of tumors. The danger of present nomenclature is that terms based on one system of criteria, for example histologic, are used to imply the possession of other properties belonging to a different category. If, for example, the diagnosis of carcinoma in situ is based on histologic criteria, the term should be used to indicate a par-

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ticular histologic structure without any of the implications about clinical behavior that are associated with the word carcinoma. Many terms in common use are inconvenient and deplorable. For the present, it seems best to name lesions by comparison with the most well-defined prototype, as, for example, carcinoma in situ of the cervix and latent carcinoma of the prostate, and use the terms in a limited and strictly defined sense. Difficulties will remain; provision must be made, for example, for a clinically manifest "latent carcinoma" of the prostate, a contradiction in terms for which there is an unfortunate precedent in "aleukemic leukemia." The latent cancers, occult cancers, regressing cancers, cancers in situ, and so on all lack one or more of the characteristics usually associated with the word "cancer," and they differ also from one another in their particular associations of unit characters.

It is recognized that some statistical correlations, as for example between clinical behavior and tumor "type" or "grade" do not hold good in individual patients. It is less widely recognized to what extent commonly used methods of investigation, reporting, and analysis obscure the course of events in individual patients and, still more, in individual lesions, and by so doing evade fundamental issues in the natural history of cancer. Moreover, no methods of investigation or analysis can give answers to unanswerable questions such as, "Is chronic mastitis precancerous?" Problems implied by questions of this kind can be solved only by breaking them down into answerable questions. The breakdown suggested by the present review is into paths of development, stages of development, and unit characters that can be studied separately. Neoplasia needs studying from beginning to end as a dynamic process advancing along many different pathways and through *qualitatively* different stages at which there is a choice between regression, persistence without qualitative change, and progression by irreversible qualitative change, involving the manifestation of properties not formerly present. It is unlikely that retrospective surveys can yield much new information about the natural history of cancer.[174] There seems no escape from the tedious and unwelcome method of prospective study and the *ad hoc* collection of new information guided by what is already known or suspected about neoplastic development as a result of clinical experience and laboratory research.

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ON THE ETIOLOGY OF CANCER

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CANCER is a popular generic term for malignant neoplasms, a great group of diseases of unknown (and probably multiple) cause, occurring in all human and animal populations and arising in all tissues composed of potentially dividing cells. Its basic characteristic is the hereditarily transmissible abnormality in cells that is manifested by reduced control over growth and function, leading to serious adverse effects on the host through invasive growth and metastasis.

THE CONCEPT OF CAUSATION

Reference to a standard unabridged dictionary or encyclopedia reveals how difficult it is to define "cause" and how many diverse meanings have been given to the word since the days of Aristotle. The philosophic and semantic complexities are equally evident in medical etiology, the study of causation of disease.

The human mind is first confronted with the effect and not with the cause. It is only after the effect is defined that reasons for (or, more correctly, events preceding) the effect are sought. The relationship between the two is then clarified through an analysis of the intermediate steps, usually referred to as the "mechanism of action," or, for disease, "pathogenesis."

There are few, if any, simple or single causes in biology. There are, instead, complex situations and environments in which the probability of certain events is increased.[11,98]

The probability of the neoplastic event following exposure of an organism to a carcinogenic stimulus is modified by a large series of factors. In regard to the stimulus, among the more evident influences are those of dose, route of exposure, physical state of the material, and length and schedule of exposure. In regard to the host, the probability of the neoplastic reaction is influenced by the genetic background, age, sex, nutritional status, and intercurrent infections. The longer the period between initial exposure and the end point of the reaction, the more opportunities occur for the introduction of additional modifying factors.

No tissue is composed of one type of cell, and cells of the same type vary in age and in their relations to each other and to the environment, as well as in such presumably basic characteristics as the number of chromosomes.[51] A completely

uniform response of cells or tissues is, therefore, a biologic impossibility. Despite the wide variations in individual responses, however, the neoplastic reactions of organisms of specific species and strains are surprisingly reproducible and suggest that the reactions are mediated through rather specific mosaics.

The production of a neoplasm following a defined stimulus does not imply knowledge of the mechanism through which such an effect is induced. The crowding of mice in a cage leads to changes in food consumption; this affects hormonal secretions, as manifested by the estrus cycle; this, in turn, is reflected in the incidence and time of appearance of mammary tumors. Sociologic and psychologic influences upon physiologic processes are obvious extrapolations, and the possible effects of these influences upon the occurrence of neoplasms should occasion no surprise.

The acceptance of a cause-effect relationship between a presumed stimulus and the eventual effect usually depends upon demonstrable connection and progression between various identified intermediary steps. Reversal of the effect at will by withdrawal of the stimulus is important evidence for a causal relationship. Establishment of cause in most biologic situations depends upon the creation of an experimental model with stringent controls. If this can be accomplished, the word "cause" and the concept it describes often disappear, just as the word is seldom encountered in reports of research in the basic physical and biologic sciences. This semantic atrophy suggests that causation is a rather fuzzy abstraction with a touch of metaphysics. Preoccupation with cause usually means that the event is ill understood; and in cancer this is indeed so.

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THE NATURE OF CANCER

It can be considered as established that cancer is a disease of the cell that is transferred to the descendants of the cell. The disease is recognized by the behavior of a population of abnormal cells within a normal tissue, as manifested by varying degrees of morphologic disorientation, aggressive growth and invasion, with ultimate destruction of the normal cell population.

The evidence that cancer is a characteristic of individual cells is based upon extensive experiments on the transplantation of tumors since the turn of this century.[121] These investigations culminated in the work of Furth and Kahn,[30] who succeeded in transmitting leukemia in mice with a single cell. With the advent of reproducible methods of tissue culture and of heterotransplantation of tumors, some cancer cells of human origin have been grown in tissue culture, carried in the tissues of a laboratory rodent,[118] and retransplanted into the species of origin,[105] with the maintenance of morphologic and biochemical characteristics. Insofar as the postulates of Koch regarding bacterial diseases can apply to dissimilar situation, the postulates have been fulfilled for cancer cells.[89]

Carcinogenesis in vitro was first convincingly documented by the research groups of Earle[22] and of Gey.[34] During recent years the ready appearance of morphologic and biologic evidences of malignancy in cells of many types grown in tissue culture has been a matter of considerable surprise.[71,78] It has been suggested that this frequent occurrence is due to cancer cells that are present in latent form in tissues and are exteriorized under tissue culture conditions. This

is a sort of latent-virus theory on the cellular level and can be safely discarded in view of the research reported by Sanford, Likely, and Earle.[92] Various cell lines were developed from a single isolated fibroblast; the clones included one line which produced sarcomas in almost all mice that were inoculated, two lines that produced no tumors, and another line that gave rise to very few sarcomas unless the mice were radiated.

Cancer is curable in man and in animals upon the total excision of the neoplasm, a fact which even such vigorous critics as Lees and Lees[70] and Jones[58] have to accept if cancer of the lip is used as an example. It is still valid support of the thesis that initially cancer is a localized process and does not involve the whole organism. Ostenfeld[84] and Goldie[35] demonstrated the presence of tumor cells in distant organs of mice within a short time after implantation with tumors, but found that this did not necessarily lead to eventual metastases. As with other biologic material, survival of cancer as a growth depends upon favorable environmental conditions and in most instances requires a large number of cells for successful growth of a tumor.[125]

Both for the stage of carcinogenesis and for the maintenance and growth of a tumor, therefore, the focal point is the cell, and not the status or even the presence of the total organismal host.

The sharp categorization of cells into normal and neoplastic, used in the foregoing for purposes of communication, can no longer be maintained. It is a carry-over of the views held 20 or more years ago,[99] when it was considered that the malignant property of the cells develops suddenly and becomes a fixed character.

It is now clear that the neoplastic process is not a single event that endows cells with immutable, full-blown malignant characters. The step-wise, slow development of many neoplastic entities in man as well as in animals has now been amply demonstrated. Greene[37] observed the progressive stages in the evolution of mammary cancer in rabbits, and Foulds[28] in mice; Furth[29] showed such stages in the evolution of several induced tumors of the endocrine system in rodents, and Berenblum[5] introduced the concept of successive developmental stages of carcinogenesis and co-carcinogenesis of the skin.

In man, the transition of rectal polyps to carcinoma and of "carcinoma in situ" of the uterine cervix to the invasive neoplasm probably represent analagous gradual acquisition of new characters by cell populations. The detection of the early stages of cancer by exfoliative cytology has developed into a practical public health procedure and has indicated the protracted period of a decade or more that may exist between the "in-situ" stage and frank neoplasia.

Thus, the concept of a sharp distinction between normal cells and cancer cells has to be replaced by a wide range of cellular potentialities, from the well-organized, self-disciplined patterns we call normalcy to the unbridled individual initiative of neoplasia.

There are additional attributes which can be applied generally to the initiation of neoplasia. The first is related to the progressive stages leading to full neoplasia. In most situations in which the external stimulus leading to cancer appears to be well understood (or accepted), such stimulation has to take place

over a protracted period. This is true of carcinogenic hydrocarbons that evoke sarcomas upon single subcutaneous injection, for the material remains at the site for a long time. However, this is not true of some neoplastic reactions; for example, the pulmonary tumor of strain A mice is evoked by a single injection of urethane. The chemical is excreted within a day's time, and the tumors seem to have practically no latent period, their recognition as tumors being dependent upon a minimal number of cellular divisions to achieve an identifiable mass.[100]

Berenblum's[5] formalization of the carcinogenic and cocarcinogenic phases of skin cancer also may indicate that the neoplastic reaction has to proceed through a series of progressive changes, and that different stimuli can be used during the early stages and in stages that complete the reaction. An alternate explanation which is difficult to dissociate is that the cancer reaction in individual cells is completely evoked by a few applications of the carcinogen, and that the cocarcinogenic stimulus acts only on the surrounding stroma so that the dormant cells can begin to proliferate. At any rate, it is accepted that once the carcinogenic reaction has been fully evoked, the further presence of the initiating carcinogen is no longer required.

Is cancer, then, the acquisition of new characteristics by a cell, or the loss of normal control factors? On the basis of presently available knowledge, both interpretations are feasible, and the choice is dependent upon the reference point of normal growth and division. If the reproduction of cells in a normal adult organism is used for comparison, cancer almost by definition represents excessive growth, although this can be the end result of decreased cellular destruction as well as of enhanced cellular division. There is no well-established evidence to indicate that the mere rate of cell division of cancer cells is regularly or strikingly higher than that of normal cells, and it has been pointed out repeatedly that many normal growth processes exceed those of most neoplasms in vivo. The fact of the matter is that cancer growth is abnormal and is clearly distinguishable at its end product from embryonal growth or adult regeneration. From this view-point, the term of de-differentiation is not applicable to cancer; it is probably more correctly a form of patho-differentiation. This, however, is no more than stating that cancer is cancer.

CARCINOGENESIS

For the practical purpose of investigation, and for the even more practical purpose of applying knowledge toward the control of cancer in man, study of the causation of cancer can be divided into the search for the *causal genesis*, or the inciting stimuli leading up to the development of malignancy, and *formal* genesis, or the factors responsible for the emergence of the cancer cell. These terms, introduced by Ewing, recently have been renamed by Kennaway[63] as the *empirical* and the *fundamental* cancer research, terms that have the defect of being too closely associated with status.

It should be apparent that all research in cancer is, by definition, applied research, and that any particular approach to the problem is determined by the training and the technical abilities of the particular investigator. Biochemists and virologists turn their attention to the intracellular aspects of the problem

for obvious reasons, just as the epidemiologist and the clinician are more concerned with the area of external stimuli. At the risk of further confusing the topic by the device of a new terminology, I would propose the division of these two aspects of etiology of cancer into the study of *intracellular mechanism* and the study of *extracellular stimuli*.

INTRACELLULAR MECHANISMS

There appear to be two types of intracellular processes that can account for the behavior of neoplastic cells, and these are the basis of the two most prominent theories regarding the cause of cancer. One is the invasion and symbiosis with the cell of a primitive form of life known as *virus*. The other is of changes induced in the genic material of the chromosomes, or *somatic mutation*.

Recently Burnet[10] presented a scholarly dissertation of these two possibilities and came to the conclusion that cancer viruses represent one rather potent means by which what is functionally equivalent to a somatic mutation can be produced. This interpretation of the virus theory is a neat resolution between the two views, but would merely place certain viruses among the hundreds of chemicals and several types of physical energy that can act as evokers of the neoplastic reaction. However, the nub of the virus theory of cancer is that the virus is an integral portion of the cancerization process: a cell-virus symbiosis in which the somatic cell behaves as a cancer *because* it harbors a virus. All other carcinogenic agents, physical or chemical, are interpreted as acting on the cells through stimulation or evokation of a more or less ubiquitous virus within the cell.[21]

It is not surprising, of course, that there are defects in interpreting cancer as a mutation or a virus parasitization. Both are attempts by analogy and by description to fit neoplasia into other known classes of biologic phenomena. Were it simply one or the other, it would have been established as such by now; but it is probably a distinct biological entity, the eventual clarification of which will allow the human mind to attack by analogy yet further undefined problems.

The recent revitalization of the investigations of viruses in cancer has been gratifying from many viewpoints. It is, in part, the natural turn of scientific attention and technical armamentarium from the victories in poliomyelitis. Its main contribution is the new optimistic spirit it has re-established in cancer research, something that was sorely flagging from the unsuccessful biochemical forays in carcinogenesis. All this is to the good, and perhaps research on virus diseases will have its greatest day in cancer. But it is dangerous to confuse hope with reality, and speculation's rightful place is being the framework for experimental design.[106]

Since the introduction of the electron microscope, a series of investigators have studied and published observations of intracellular particles in a wide variety of neoplasms. Dmochowski[15] has recently reviewed the topic. As in the earlier days of bacteriology, electron microscope findings can be evaluated properly only if combined with suitable biologic tests of the material. Thus there is a firm scientific place for the particles isolated in fowl leukosis[3]; these are virus in every necessary definition of the term. Of more than passing interest is that even in this clear-cut viral entity the virus endows itself with some im-

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munologic properties of the chicken host. On the other extreme of unproved, albeit suggestive, observations are such as those of Leuchtenberger,[72] who reported cytoplasmic inclusion bodies containing desoxyribose nucleic acid in 63 of 63 human rectal polyps, whereas 113 specimens of other kind failed to reveal such particles. Perhaps tissue culture methods recently used by Stewart and associates,[112] combined with cortisonized laboratory rodents, or erythrocyte adsorption[126] may yield methods that will extend these morphologic findings to an understanding of the biologic implications.

Cancer research, as all human endeavor, has its fashions, which upon being replaced by still other fashions appear as ludicrous as outdated female apparel. Some twenty years ago opinion was developed of exquisite specificity of tumor cells and viruses: tumor cells could be transplanted only into homozygous recipients, and viruses were able to parasitize only specific cells in specific species. Duran-Reynals[20] first showed that the Rous sarcoma virus could overcome species differences and could be transmitted from fowl to ducks if large, intravenous doses of virus into newly hatched ducks were used. In the new host, the virus behaved somewhat differently and produced bone tumors as well as a hemorrhagic lesion. It has been known for many years that resistance to heterologous tumor transplant could be decreased by x-radiation and by injections of trypan blue. Heterotransplantation was more recently reintroduced and its potentialities recognized through work with human tumors, as reported by Toolan.[117] It should be emphasized that such transplantation occurs with only a small proportion of tumors and is probably no more representative of neoplasms of the species than the few active, transplantable neoplasms of rodents that have led to so much generalization by biochemists who appreciated their ready availability and convenient behavior.

There is a rather impressive list of neoplasms in animals in which a virus entity plays a causal role. The avian virus tumors have been used effectively in quantitative studies by Beard and his collaborators,[3] and by Bryan.[9] The Shope rabbit virus[90] and the Bittner mouse virus[6] have been useful materials for many engrossing biologic problems of tumor-virus relationships. It is now clear that mammary tumors in mice appear in the absence of demonstrable agent. Latency or masking can be brought to the fore as explanation, but these phenomena can be established only by a demonstration of activation or unmasking.

The most interesting developments in the virus studies on cancer have been reported by Gross[39-41] and by Stewart.[110-112] It is only proper, with the verification of the basic work by several investigators,[28A,111] to designate as the Gross agent or virus the filtrate of leukemic mice that on injection of newborn mice leads to the appearance of leukemia. The material produces not only leukemia but parotid gland tumors, sarcomas, and adrenal gland tumors. Stewart and her co-workers[22A]. have gone further in destroying the concept of specificity by producing a variety of tumors in hamsters injected with supernatents of tissue culture in which mouse tumors were introduced.

These researches may have connection with the observations of Schwartz and his group,[94] who report the earlier appearance of leukemia in mice following introduction into the animals of cell-free filtrates of tissues from human patients

who died of leukemia. Perhaps the combination and exploitation of techniques already available may establish the viral etiology of some human neoplasms. The hopes of diagnostic reactions and therapeutic and preventive possibilities that would then be opened are abundantly evident.

EXTRACELLULAR STIMULI

The pre-World War II decade was an optimistic one in cancer research: almost any investigator could produce a galaxy of tumors and publish well-illustrated papers on pure, potent chemicals with beautiful fluorescence spectra. Kennaway[61] has recorded the events of this major discovery of polycyclic carcinogenic hydrocarbons, in a paper that will remain a medical historical classic.

It seemed to be a reasonable hypothesis in those halcyon days that a specific chemical alteration of the cholesterol molecule produced a common-denominator carcinogen in the body, and an important encouragement was given to this concept when 20-methylcholanthrene was synthesized from bile acids. The thesis of specificity was soon found to be untenable, with the rapid addition of a series of azo dyes, estrogens, aminofluorenes and even such simple chemicals as carbon tetrachloride to the list of carcinogens. In the second edition of his monograph, Hartwell[46] records 322 compounds which have been reported to cause malignant tumors in animals, and the supplement just issued adds 150 more.

Of the newer classes of chemical materials that have demonstrated carcinogenic properties following subcutaneous implantation in mice and rats, the synthetic plastics and polymers[82] are among the most interesting.

In 1937, Shabad[96] reported that benzene fractions of human liver produced sarcomas in mice following subcutaneous injection. Hieger[48] has now established that cholesterol is a weak but positive carcinogen in mice, and the theories of carcinogenesis of twenty years ago, now discarded as naive, may bear re-examination. As Kennaway[62] suggests, a search for weak carcinogens and cocarcinogens in human tissues is definitely a desirable area of research that deserves long-term support. Smegma, contents of dermoid cysts,[87] and scars of thermal burns,[69] are good candidate materials for the search of endogenously produced carcinogens.

In 1946, Dunning and Curtis[19] reported that sarcomas in rats can be produced not only by infestation with the cat tapeworm larva, *Cysticercus crassicolis*, but also by crude fractions of the parasite. Further chemical curiosity in determining the nature of the carcinogen was not stimulated and still awaits exploitation. Even less attention has been accorded to the very important problem of *Schistosoma hematobium*, a trematode infesting man, which also harbors a possible carcinogenic fraction.[54]

For completeness, reference should be made to the carcinogenic potentials of two forms of physical energy, ionizing radiations and radiations in the ultraviolet range. There has been little interest in the latter since the publications of Blum and his group[7] of a decade ago. It is commonly accepted that actinic rays can induce carcinomas on the exposed surfaces of human skin, particularly in races deficient in skin pigment.[1] A re-study of the epidemiology of this topic may not be amiss, since the diagnosis of skin cancer is now more often substantiated by biopsy.

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Ionizing radiation from external sources or in the form of fission products continues to reveal an ever wider carcinogenic spectrum as more species of animals are studied at longer periods following exposure.[33,86] Mice develop pituitary, thyroid, and other tumors of the hormonal system, which Furth[29] has studied meticulously, as well as the earlier neoplastic reactions such as leukemia. In man, thorium in the form of thorotrast leads to the induction of hemangiosarcoma of the liver after a period of many years,[101] and x-rays externally delivered in therapeutic doses and schedules have produced osteogenic sarcomas in apparently normal bone.[91]

The field of chemical carcinogenesis has been reviewed systematically by Greenstein,[38] and by Haddow,[42,43] and many other authors have undertaken synthesis in more limited aspects, such as in hormones[32,98] or in azo dyes.[76] Haddow[43] particularly has presented scholarly dissertations of the possible modes of action of certain classes of carcinogens.

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Five years ago, Goldblatt and Cameron[36] reported that intermittent anoxia of rat fibroblasts grown in tissue culture led to the development of malignant changes. The work has been criticized, perhaps correctly so. What is more surprising, however, is that there has been no systematic attempt to repeat and to extend the investigation. Certainly a clearer knowledge of the effect of anoxia and of pH and the development of neoplastic characteristics in vitro is more than of passing interest. Even if it were shown that this is the common-denominator carcinogenic stimulus, as suggested by the theories of Warburg,[119] there would still be need for clarification of the mechanism that then translocates genic material, activates a cytoplasmic plasmogene, unblocks enzymes concerned with protein synthesis, or destroys enzymes controlling such synthesis.

Attempts continue to be made, particularly by the Pullmans,[88] to relate carcinogenic activity and the physical and chemical properties of the molecules. As with other attempts in biochemorphology, correlations are reasonably satisfactory within fairly narrow groups of compounds. However, such theoretical work should be encouraged, if for no other reason than that it focuses on the need for better biologic data. Into this general category also enter the interpretations of Armitage and Doll[2] and of Iversen[56] of the mortality data on cancer as a series of mutational hits on the population of some 10^{13} cells that comprise the adult human body. The formulations of theories as models,[83] as well as the testing of models using available data, have practical uses for further research as well as intellectual stimulation.

The neglect in these comments of the role of heredity in carcinogenesis is deliberate only because it has been well covered in several previous reviews.[47,68] Koller[65] recently summarized and evaluated the subject in an admirable fashion.

ENVIRONMENTAL CARCINOGENESIS

Percival Pott's account of chimney-sweeps' cancer of the scrotum, published in 1775, will remain the first immortal observation of environmental carcinogenesis. Several additional carcinogenic situations in man have been discovered in which the neoplastic induction is as convincing as in a group of inbred mice injected with methylcholanthrene. Bladder cancer in workers in the dye indus-

try, traced to β-naphthylamine, and osteogenic sarcoma in girls who ingested radium in the process of preparing luminous watch dials are sufficient examples.

Somehow, it became accepted that these instances of human carcinogenesis were limited to industrial or occupational hazards. This comforting rationalization was supported by a somewhat fatalistic scientific attitude of attributing neoplasms to a predetermined occurrence of somatic mutations. It is now clear that there are many important carcinogenic situations to which human populations outside of industrial environments are liable. Among these, two groups of carcinogens are of paramount importance: products of incomplete combustion of carboniferous matter, and ionizing radiations from various sources.

Most of the environmental carcinogenic situations that have been analyzed adequately concern materials which exert their effect on direct contact and lead to the appearance of skin cancer. This is due to the obvious fact that this type of reaction is the easiest to detect and to follow. It can be taken for granted that the respiratory and the digestive routes, the other main means of contact between man and his environment, will be found to be more important than the skin exposures. This is already becoming evident for materials that gain access to the body with the inspired air, where the neoplastic reactions may take place not only at the first area of deposition but in distant tissues to which such materials may be carried. The great undeveloped field in the study of environmental carcinogenesis is the role of ingested materials. So far, there is disappointingly little reliable information regarding dietary relationships in the major forms of cancer of the gastrointestinal tract. It is probable that new experimental methods will have to be devised to measure accurately the unreliably remembered dietary habits of man. In this connection, the introduction of chemical additives to foods has been recognized as a potential problem,[116] and requires thoughtful, objective study.

The thesis of environmental carcinogenesis is so attractive that care must be exercised constantly not to derive premature conclusions from inadequate data. Hueper[52,53] particularly has written at length regarding possible carcinogens with which modern man comes in contact.

Essentially two lines of evidence are needed in establishing an agent as a carcinogen for man: a carefully controlled epidemiologic study in a defined human population, preferably exposed to graded dose levels of the material, and laboratory demonstration of carcinogenic activity in animals. The need is for long-term, large-scale investigations in which small increases of tumor incidence can be detected[62]; insistence upon rapid, obvious results with isolated, defined chemicals is a restrictive concept of the methylcholanthrene era in cancer research.

It is difficult, of course, to indicate the exact point at which data become adequate to establish that a certain agent or situation lead to an increased or earlier appearance of cancer. A limited number of such situations will serve as illustrations for further search and practical application: these include an industrial cancer, a sociobiologic cancer, and a habit cancer.

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CANCER OF THE SCROTUM

Cancer of the scrotum is an example of an industrial cancer that illustrates a principle of wider application in environmental carcinogenesis. The Kennaways,[84] in 1946, summarized the cumulated figures on mortality from cancer of the scrotum in England. Among 5,274 chimney sweeps, 118 such cases were reported. Other occupations that are also heavily exposed to soot, tar, and mineral oils, such as cotton mill spinners, gas, tar, pitch, and creosote workers, were found to have 529 deaths among 189,650 men. Occupations with only occasional contact with these agents were represented by 306,348 men, and these had 76 deaths from scrotal cancer. In 289,985 individuals of 17 professions not known to come in contact with soot and tar, there was only one case of scrotal cancer as a cause of death, and this occurred in a man with a history of possible occupational exposure.

Although chimney sweeps' cancer has been accepted as an industrial entity for almost two centuries, its implications thus extend into the general population. The laboratory production of cancer with repeated applications of tar was achieved in 1915, by Yamagiwa and Ichikawa. It should be pointed out that very few investigators have applied tar on animal scrotums, that soot as a source of carcinogens rather than tar was finally used almost as an afterthought by Passey,[85] and that is is still far from clear whether 3,4-benzpyrene isolated by Kennaway's group is the only or the chief carcinogenic chemical in either tar or soot.

Nevertheless, whatever eventual "cause" of cancer may be accepted, it is generally agreed that tar and soot products have a role in the occurrence of scrotal cancer in England, and that this particular form of cancer could be practically eradicated by some direct, obvious measures of public health and personal hygiene.

Of course, it is possible to conceive that the occurrence of scrotal cancer among chimney sweeps is the result of selection; men with genetic proclivity of developing scrotal cancer choose chimney-sweeping as their faviorite occupation, and the soot and tar are statistical artifacts. This could be claimed to be supported by the fact that only a very small proportion of chimney sweeps die of cancer of the scrotum, and cancer of the penis and perineum are not produced, although these sites are also exposed to the purported carcinogens.

Additional mental legerdemain may lead to the conclusion that nothing should be done about the problem of scrotal cancer in chimney sweeps until all steps in the carcinogenesis of skin are thoroughly understood, and indeed, that there should be no advertisment of the findings to the chimney sweeps, since they seem to be happy, and a change in habits may lead to obesity.

It is interesting that the factors leading to cancer of the scrotum do not apply to cancer of the penis, although both are classified as epidermoid carcinomas. The prominent feature for penile cancer is the rarity of its occurrence when circumcision is performed early in life.[60,93] The role of some constituent of smegma as a carcinogenic stimulus has been strengthened by the production of cancer in mice painted repeatedly with smegma.[87]

CANCER OF THE UTERINE CERVIX

The occurrence of epidermoid carcinoma of the uterine cervix appears to be influenced by the sexual habits of the female and male. The early observations on the infrequency of cervical cancer in Jewish women have been substantiated by more recent, controlled epidemiologic studies in New York and in Israel.[17A] That this is attributable to environmental habits which are becoming less rigidly adhered to, rather than being due to purported racial or hereditary traits, is strongly suggested by the gradual decrease in the relative disparity of the occurrence among non-Jewish and Jewish women. In New York, this relative frequency has steadily decreased from 17 to 1 at the turn of the century to a ratio closer to 5 to 1 now observed.

In a detailed retrospective study, Wynder and associates[124] have described positive association between cancer of the cervix and the following sexual traits: (1) earlier first coitus and first marriage, (2) greater number of multiple marriages, and (3) more frequent sexual exposure to uncircumcised males. None of these findings was more than twice as frequent as in the comparable controls, so that the associations were of relatively low order. A statistical association with syphilis was attributable to the longer, more active sex life in patients with cervical cancer. No association could be shown with pregnancy, abstinence after menses, douching, or the presence of irritative discharge. The frequency of cancer of the cervix has been reported as being extremely low in nuns,[31] and four times as numerous among prostitutes than among other women of comparable socioeconomic groups. It would be of interest to determine the distribution of cervical cancer in women who confided about their sexual habits to the late Alfred Kinsey.

Insofar as practical measures are concerned, the association of noncircumcision with the occurrence of cervical cancer is the most obvious. Circumcision becomes sensible with the evidence of an association between noncircumcision and penile cancer, and of the experimental data on the production of neoplasms with smegma. It should be pointed out, however, that the relationship between cervical cancer and noncircumcision has been reported in only one investigation and certainly needs confirmation and further study before it is accepted as anything more than an interesting lead.

As with every type of neoplasm, carcinoma of the cervix is undoubtedly the result of multiple etiologic factors. Further study undoubtedly will uncover additional factors which may lead to means by which the frequency of this lesion can be reduced.

At the present time, however, cervical carcinoma represents a challenge to early case-finding as a method of controlling mortality from one type of neoplastic disease. The examination of exfoliated cells from the vagina will detect the neoplasm at a biologically early stage. Perhaps the worst disservice to the smear diagnosis of cancer has been the *term* "carcinoma in situ." It introduces arguments that are insoluble and unnecessary. On the skin of hands, precursors of carcinoma are called keratoses, and everybody seems content with the term. The pressing problems of substance are to make the cytologic examination available to all women in a constant, periodic fashion and to determine how to treat women found to have atypical cellular elements. The latter should be

approached as a designed therapeutic trial, with randomized assignment of women to various forms of management. The long period that on the average occurs between the noninvasive and the invasive stages of the neoplasm allows observations of this type with little risk to the patients. Such a trial should yield with maximum economy the best method of treatment for an important lesion with a curability rate that should approach 100 per cent.

Unlike scrotal cancer, which is a rare disease, cancer of the cervix is a numerically important entity: in 1947, the incidence in the United States was recorded as 44 per 100,000 women and was exceeded only by breast as the most important single site of cancer.[18]

CANCER OF THE LUNG

Studies on the relationship between smoking, especially of cigarettes, and epidermoid-undifferentiated carcinoma of the lung have been so extensive and convincing, since 1950, that they allow no other conclusion but that smoking is one of the causes of this neoplasm. The evidence is now applicable to women[122] as well as men.[16]

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The prospective studies by Doll and Hill,[17] and by Hammond and Horn,[45] on the relationship of smoking to death rates, are, in effect, now complete, and the third large study on United States veterans of World War I will be heard from shortly. All evidence continues to demonstrate the greatly increased risk of cigarette smokers to lung cancer. In the 44 months of the Hammond-Horn study of 187,000 men, the following figures are derived from the occurrence of carcinoma of the lung with well-established diagnosis and excluding adenocarcinoma: among 14,136 rural nonsmoking men, there were no deaths from this cause; among 23,560 rural men who smoked cigarettes there were 52 such cases. Among 8,481 nonsmoking men living in larger cities, there were 4 lung cancers, as compared with 83 lung cancers among 28,270 urban men who smoked cigarettes. These figures reveal the factor of urbanization, presumably atmospheric pollution, as well as the factor of the individual self-induced smog of smoking.

In the laboratory, it has been demonstrated by at least five independent investigators that condensates of smoke of tobacco products induces carcinomas of the skin in mice.[113] Whatever the chemical nature of the carcinogens and cocarcinogens may be eventually elucidated, the agents are present either in very small concentrations, or are weak in activity, since certainly the neoplastic response is not easy to elicit.

The conclusion of a cause-effect relationship between lung cancer and cigarettes has been widely accepted by national and international bodies of scientists.[74,113,114] A recent hearing on the topic of filter tip cigarettes before a Congressional Committee[50] marshals much of the pro and con material; the nature of the testimonies should be read in the original to be fully appreciated.

The conclusion of a causal role of smoking in cancer of the lung does not imply that all problems and questions regarding the mechanism are solved. Indeed, it opens up some intriguing facets of epidemiology and experimental pathology, such as why the male-female ratios among Mexicans in the Los Angeles area are closer to unity than elsewhere[5A], or why the trachea is so immune to carcinogenesis by chromite and other carcinogenic materials as well as tobacco

tars. Nor does it suggest that smoking is the only cause of lung cancer, since at least five other materials that gain access to the lung have been established as being carcinogenic[23]: pitchblende, coal-gas, chromite, asbestos, and hematite, and there are probably dozens more in the field of environmental inhalants alone. Moreover, the conclusion of course does not indicate that research in cancer should now be directed only toward smoking; quite the reverse, the data have opened new approaches and should lead to accentuation upon a broad front of research.

In my opinion, however, the conclusion definitely *does* demand that the fruits of science be recognized and be used for the good of mankind. The public health goal is obvious: cigarette smoking should be discouraged because it is a health hazard. The practical methods to be used toward the goal are not quite so simple. It would be an unrealistic position to suggest prohibition of the manufacture or the sale of cigarettes. For one thing, this would not work. On the other hand, the obverse position that nothing can or should be done is just as untenable. It has been reiterated *ad nauseum* that the growing consumption of cigarettes in the face of information to the public regarding the increased cancer risk is evidence of the immutability of human habits. Announcements of the scientific findings do produce temporary depressions of a few per cent in the sales, a phenomenon that at least the tobacco industry does not take lightly. Occasional health information announcements have to compete with billboard, magazine, newspaper, radio, and television hucksterism of the most expert sort, directed at youngsters, and cleverly identified with pleasure, social acceptance, sexual attraction, and manly vigor. An examination by appropriate representatives of government, industry, and education of the role of public health education and of regulation of advertising over public media would appear to be legitimate first steps in the proper direction.

At the same time, there is need for additional studies of the urbanization and occupational factors in lung and other cancers, and for the control of atmospheric pollution from individual as well as industrial sources. This problem is found in Leningrad[14] as well as in Los Angeles,[66] and its importance is of first rank.

Cancer of the lung has been likened to a pandemic in its rapid universal increase throughout Europe and America. It is now one of the major forms of cancer in men[18]; in the United States in 1947, the incidence was 29 per 100,000 men and 6.5 per 100,000 women, and this has risen during the past decade. Over 25,000 deaths were attributed to this cause in 1955. The control of environmental carcinogens leading to lung cancer may yield additional dividends in reducing the occurrence of other neoplasms as well. Carcinogens that are inhaled are also swallowed, so that the gastrointestinal tract and the excretory organs[13A] also may be exposed to neoplastic hazard.

IONIZING RADIATION

Ionizing radiations, along with the chemical pollution of the atmospheric and alimentary environments, represent an important source of carcinogenic exposure for the populations of the future. It is instructive to recall that the

first carcinogenic results of x-rays, on the skin of the hands, were reported within 7 years after Roentgen's discovery, but that 50 years later neoplastic effects in various internal tissues exposed to very small doses are still being found in laboratory and in epidemiologic research.[33,72A]

The Hiroshima experience has yielded conclusive data on the leukemogenic effect of ionizing radiation.[77] Several studies have been recorded on the neoplastic potential of much smaller exposures, including the range of doses for diagnostic radiologic procedures. Court-Brown and Doll[12] showed that the occurrence of leukemia in 13,352 patients irradiated for ankylosing spondylitis was increased tenfold over the expected (28 cases as compared with 3). Simpson and his colleagues[102] showed that among 1,400 children who were treated with x-ray for thymic enlargement there arose 7 leukemias and 6 cancers of the thyroid; among 1,795 siblings there were no instances of these two neoplasms. Stewart's[109] survey of children who were irradiated in utero as a result of pelvimetric radiologic procedures on the mother showed a twofold association between leukemia and such exposure, as compared with a control group. Careful consideration of these and other data indicates the incomplete nature of the investigations; expansion of research in this area is mandatory.

The statement by the United Nations Scientific Committee[118] on the effects of atomic radiation spells out the medical responsibilities in the use of x-rays and other ionizing radiation. It is an interesting world of relative risks and values indeed when, on the one hand, we recommend the reduction of exposure to diagnostic radiologic procedures, and on the other hand accept that the explosion of atomic devices unfortunately must continue.

OTHER ENVIRONMENTAL SITUATIONS

The few examples indicated above are perhaps sufficient to indicate the broad scope and the rich potentials of investigations in environmental carcinogenesis. The human mind, like the eye of the dog, is attracted by motions and contrasts. For this reason, research has been most prominent on neoplastic diseases that show significant differences in geographic, occupational, or some other aggregations, or that can be demonstrated to have altered in incidence with time.

Cancer of the larynx[63,123] and of the esophagus[79,103] demonstrate sharp differences in occurrence among groups of different socioeconomic status. Both show association with the use of alcohol and of tobacco. Cancer of the stomach, a topic recently reviewed by Haenszel,[44] also shows socioeconomic gradients and again suggests exploration of the dietary factors. It is interesting that in the United States and Western Europe this particular neoplasm has shown a drop in mortality during the past several decades, a finding that cannot be accounted for by changes in registration or by therapeutic triumphs.

In contrast with these "mobile" neoplastic lesions, some neoplasms have shown little progressive change in incidence or distribution among the population. Carcinoma of the breast is an excellent example; aside from a somewhat higher rate among nonmarried, nonparous women, and a minor, temporary change in rate at the age period of menopause, its occurrence and deaths therefrom have

remained embarrassingly constant for an incontroversial defense of surgical curability. The most interesting epidemiologic finding is the low incidence of breast cancer in Japan.[95]

This brings us to the matter of geographic pathology, one of the richest fields of inquiry on cancer that can be cultivated. Even a cursory examination of the transactions of the fifth meeting of the International Society of Geographic Pathology[55,115] cannot help but impress with its potentials and the missed opportunities. Schistosomal cancer of the bladder in Africa has had pitifully little experimental or epidemiologic attention.[54] The high frequency of gastric cancer among the Japanese,[95] of nasopharyngeal cancer among the Chinese,[103] of liver cancer among the Bantu,[81] to mention a few better-established situations, are compelling challenges to research.

CANCER AND OTHER DISEASES

An interesting source of information on the etiology of cancer that has not been fully or satisfactorily exploited is the association of cancer with other diseases. This is a tricky statistical field, as Wilson[120] showed of Pearl's erroneous claim of a negative association between tuberculosis and cancer.

There are two types of such situations. In the first, the noncancerous lesion and the neoplasm occur in the same tissue or organ. If a high clinical association is demonstrated, such lesions are often termed precancerous. The abnormal epithelium of the uterine cervix designated as "carcinoma in situ" is a typical example, although it is not too well known what proportion of these lesions eventuate in invasive cancer. Related problems of this type are the role of gastric ulcer as a possible precursor of gastric carcinoma or of thyroid nodules as precursors of thyroid carcinoma.[104] The association of these lesions is hard to accept without an actual experiment on patients designed to answer the question, since on usual clinical material it is impossible to prove, for example, whether the gastric cancer that supposedly arose from an ulcer may not have been in fact a slowly growing ulcerated carcinoma. This difficulty is also not entirely obviated in the reported relationships of cirrhosis as a precursor of primary carcinoma of the liver.[73] Increased risk to cancer of the rectum seems to occur in individuals who recover from chronic ulcerative colitis,[49] as well as in familial polyposis,[27] to cancer of the esophagus in women with Plummer-Vinsen syndrome,[79] and to cancer of the stomach in patients with pernicious anemia.[59]

The second type of relationship worth more attention is between apparently unrelated diseases, at least insofar as involving the same organ or tissue. Among these situations are the frequent association of Mongolism and acute leukemia,[67] of acanthosis nigricans and carcinoma,[13] and of dermatomyositis and carcinoma.[97] The latter may be particularly significant if our understanding of dermatomyositis as one of the diseases of allergic response is correct, for it may be a lead toward tumor antigens that could elicit such a response.

Negative associations, or demonstration of no association in many instances would also be instructive. Example of such studies includes reports that arteriosclerosis is seen less frequently in patients with cancer than among controls[25]; these data need thorough scrutiny, particularly since arteriosclerosis is much more

reversible than has been commonly assumed. Diabetes mellitus does not have much demonstrable influence upon the development of neoplastic disease.[57] The incidence of cancer is about the same among psychotics as in the general population.[24]

Many studies on human beings that were previously beyond reasonable capabilities have now become feasible. This is due to the development of medical insurance schemes and other social patterns that allow easier, longer, and more accurate follow-up of patients, and of centralized medical record systems with mechanical appliances that allow automatic sorting of records.

It would be valuable to collect information on women who had been ovariectomized ten years ago, and to compare the incidence of various neoplasms in this group with a group of matched controls. Informative data could be developed in comparing the incidence of cancer in patients with opposing hormonal states, such as acromegaly and hypopituitarism[80]; with Addison's disease and hyperadrenalism; and with hyperthyroidism and myxedema. Other probands of interest, including patients who received protracted treatment with hormones,[4] would readily occur.

CONCLUSIONS

The only excuse that can exist for adding to the medical literature another paper of generalizations on cancer is to stimulate further research. It would be a disservice to suggest that any legitimate scientific approach to the uncompromising problem of cancer is erroneous. Yet the total approach may well be unbalanced. At the present time there is too small a portion of research being devoted to the etiology of cancer.[75,107] Development of such studies through the rekindling of interest and availability of support should be achieved not at the expense of other research but as a natural growth of the total research effort.

Studies on the causation of cancer include epidemiology, in the wider sense of a close partnership of field investigation, clinical observations, statistics, and laboratory work.[8] The proper sphere of epidemiology is wherever man is, and the limitation of studies of national boundaries cripples the real potential of the science. Correctly designed and executed biometric studies on human populations will yield information of practical import to man, as well as provide leads for laboratory investigations of theoretical importance. The development of the epidemiology of cancer, including geographic pathology, should approach in scope and in imagination the recent development of research on the chemotherapy of cancer.[26]

The broad view of our understanding of the intriguing biologic problem of such great practical, immediate import to man yields definite reasons for optimism.[99] Progress is slow, and a complete, all-inclusive "answer to cancer" is probably unreachable. But some partial answers applicable to specific types and sites of cancer are already available and await application. At the same time, further expansion of research on the causes of cancer is a thoroughly sound investment.

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SOME ASPECTS OF VIRUS-INDUCED TUMORS

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"NO ONE can dispute the fact that viruses are involved in the cancer process. The virus theory no longer elicits violent reactions from its opponents, and its proponents in this publication have conceded that viruses may not be involved in all forms of cancer. The theory has attained a high level of respect in cancer research, for it enables the investigator to design an intelligent approach to the many problems to be confronted. Those who pursue this course objectively are certain to contribute to the control of the disease; for it is no longer a question of whether viruses induce tumors, but in how many neoplastic processes of different species they are involved."

Andervont[1] concluded a conference on "Sub-cellular Particles in the Neoplastic Process" with these words—a most succinct appraisal of the present situation with respect to viruses and cancer. In the last few years a number of most comprehensive reviews has been published.[9,18,35,85] It is not intended that this should be just one more to add to that list. Instead, we propose to consider the more recent work with respect to avian tumor agents, and then the possible implications for the alleged virus etiology of mouse leukemia arising from the phenomenon of acquired tolerance of one species to tumor-producing agents from another. This is admittedly selective, but will, it is hoped, be stimulating to those who wish, in Andervont's words, to "design an intelligent approach to the many problems to be confronted."

THE STRUCTURE OF THE ROUS AGENT

Electron-microscopic.—Gaylord[48] was the first to obtain good evidence for the presence of Rous agent in chicken sarcoma sections. Two tumors out of the three examined contained these particles which were more numerous in the youngest tumor occurring singly, in pairs or in small clusters, usually on the outer surface of the cell membranes. Osmium staining revealed uniform, round, dense particles with thin outer membranes. The over-all diameter was about 50 mμ and the centers were very dense. Occasional particles appeared to have lost the central, dense mass, but this may well have been the result of sectioning.

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They were noticeably smaller, and it was hardly to be expected that all particles in the cell would have been cut in the same plane.

These observations were substantially confirmed by Bernhard, Oberling, and Vigier[10] who examined a far larger number of tumors (75 sarcomas, 7 ectodermal lesions on the chorioallantoic membrane, 8 liver hemorrhagic lesions, as described by Duran-Reynals,[84] and also 7 pellets produced by centrifugation of tumor extracts). The characteristic particles present in variable quantities in 63 per cent of the specimens examined had a minimum diameter of 70-80 mμ. They were bounded by an external membrane, as described by Gaylord, but the dense, osmiophilic central "core" of diameter 30 to 40 mμ was separated from the outer membrane by a second (Fig. 5). The outer membrane was more electron-dense than the inner.

Bernhard and associates were unable to show any relationship between particle count and infectivity, but such has been claimed by Epstein.[40]

Oberling, Bernhard, and Vigier[86] concluded that by analogy with the Bittner mouse mammary tumor agent, where the outer membrane is formed by a small portion of the cell membrane at the moment when the virus body segregates itself from the cell in which it originates, the Rous agent might have peripheral host antigen. This postulation would account for the structure of extracellular virus and also for intracellular if one assumes that "internal" host membrane material surrounds the cytoplasmic vacuoles within which Epstein[39] locates the virus. There is one difficulty about such a structure. Unless one makes the further assumption that the antigenic composition of internal and external tumor cell membranes is in some way characteristic of "virus" (see Harris[65]), the precipitation and neutralization of the agent by hyperimmune sera[62] become difficult to explain. The logical structure should have external, accessible "virus" antigens.

Chemical and Immunological.—Attempts to study the chemical properties of Rous agent "concentrates" have been roundly condemned by Beard,[7] largely on the grounds that the appearance of *their* electron micrographs shows that the virus content of "purified" preparations is only of the order of 1 per cent. Sharp and Beard[96] suggest "the complete lack of value of analyses on any material thus far described with respect to interpretation of the physical, chemical and quantitative biological properties of the agent of Rous sarcoma."

Fig. 1 is an electron micrograph of purified Rous agent prepared by Cuckow and Harris.[32] It will be apparent that in this air-dried preparation the particles have collapsed. The shadow indicates that they are discs and not spheres, but the volume of each disc is consistent with the size of the virus as calculated from other data. This is an unselected field, and the homogeneity is much greater than that shown by Sharp and Beard. The infectivity of the agent was 2×10^7 minimum infectious doses per gram of original tumor, but without making unwarranted assumptions about the number of virus particles constituting one minimum infectious dose one would not wish to state whether the field shows 1, 10, or 100 per cent virus.

The purification problems associated with the preparation of Rous agent have been reviewed by Bryan and Moloney.[20] The first approach suggested was to increase the virus concentration in the tissue. Epstein[40] studying a Rous

ascites tumor calculated that a "weak" tumor strain showed only 1 particle-containing cell in every 3,000 whereas a "strong" line had a ratio of 1 to every 57 cells examined. Bryan and Moloney's[20] "purest" preparation had a calcu-

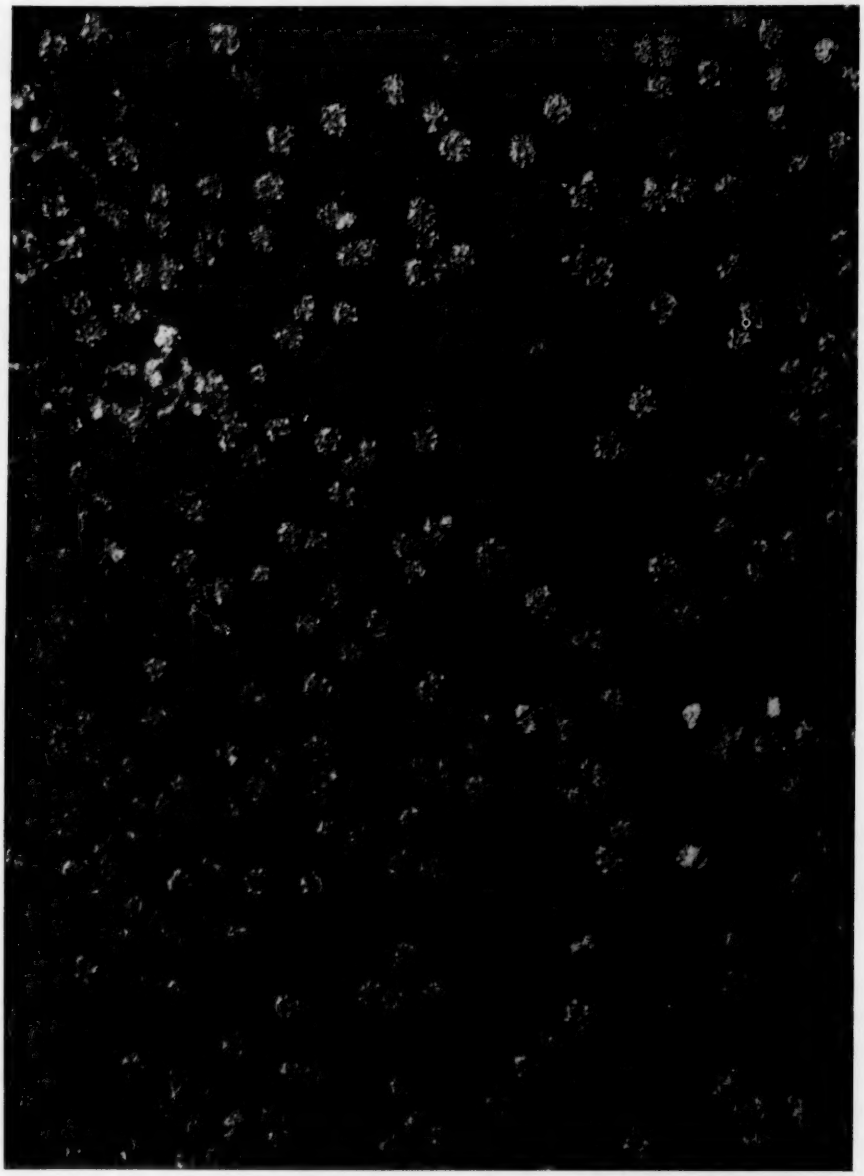

Fig. 1.—Purified Rous agent preparation, air-dried and shadowed. (Magnification x 20,000.)

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lated 630 particles for a 50 per cent infective unit. Rubin[91] who assumed, among a number of assumptions (*vide infra*), that one biological infective unit was equivalent to one physical particle, calculated that each tumor cell contained a maximum of 30 virus particles at any one time. This particular assumption, as Bryan and Moloney[20] have pointed out, ignores the plating efficiency factor which could well lead to considerable error—perhaps one thousand fold. The second approach was the investigation of the lability of the virus to prevent losses during purification, and the third, to study the degradation of nonvirus impurities. Carr and Harris[27] recommended the use of hyaluronidase as a preliminary step in the treatment of tumor extracts, followed by trypsin to degrade nonvirus protein impurities. It is discouraging, however, to find that Bryan and Moloney believe that at 37° C. trypsin may destroy the activity of the virus. Commercial trypsin-containing powders may well do so, but the original recommendation of Carr and Harris was to use crystalline trypsin.

We will now consider some of the "worthless" information about the biological and chemical properties of Rous agent which has been obtained by the study of "purified" virus. Beard and his collaborators are tilting at windmills to suggest that the analytical data themselves have any absolute value. No one has suggested that they have. When, however, we bring together the information obtained by electron-microscopy, immunology (including acquired tolerance), and biochemistry a "picture" of Rous agent begins to emerge. This may have no absolute value except as a model upon which to base future work.

The electron-microscope shows a small round, osmiophilic "core" of 30-40 mμ in diameter surrounded by a double membrane, the outer one being more osmiophilic than the inner. The studies which have been published on the substructure of rat liver microsomes[88] and our own observations on the disintegration of purified Rous agent preparations by detergents[64,67] have lead to the following conclusions. Whereas the "Palade granules" liberated from rat liver microsomes appear to contain 80 per cent of the original pentosenucleic acid, 20 per cent protein and about 10 per cent total phospholipid, the sedimentable particles (105,000 Gm., 180 minutes, Spinco Model L) from disaggregated Rous agent preparations contain about 20 per cent of the protein of the original microsomes, 45 per cent of the lipid phosphorus, and less than 10 per cent of the total pentosenucleic acid. When the deoxycholate-treated agent concentrates were dialyzed without centrifugal separation of smaller particles soluble nucleotides diffused out, a lipid-containing protein precipitated, and the nondialyzable supernatant had the properties of a nucleoprotein containing 50 per cent pentosenucleic acid.

Bryan and Moloney[20] have already commented that there appears to be too much pentose-nucleic acid associated with agent concentrates, and it is worth noting that nucleic acid is readily released from such material. Thirty per cent of the total pentosenucleic acid is released on standing overnight at 0° C. in isotonic sucrose. In 0.3 per cent deoxycholate 90 per cent of the pentosenucleic acid is solubilized, and at this detergent concentration infectivity is lost and only 1 to 2 per cent of the pentosenucleic acid is left as "intact" virus. Infectivity is lost more rapidly if borate ions are present in the medium.

These results per se probably have little significance for the structure of the virus since the "microsomal" components of normal chicken liver and of 10-day-old whole chick embryos appear to behave in a very similar manner. One observation may be made at once, however, and that is that it is highly probable that the agent has a *similar* structure to that of the normal components. Agent preparations differ, nevertheless, in two important qualitative respects, first, in the presence of infectivity, and second, in the presence of virus antigen.

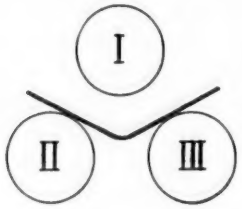

Fig. 2.

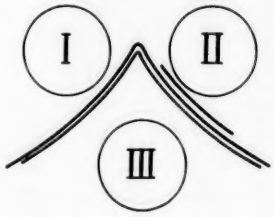

Fig. 3.

Fig. 2.—Diagram from agar plate: I. deoxycholate-treated Rous agent; II. horse anti-Forssman antiserum; III. hyperimmune chicken serum.

Fig. 3.—Diagram from agar plate: I. rabbit antifowl antiserum; II. rabbit anti-Rous sarcoma (cytoplasm) antiserum; III. deoxycholate-treated Rous agent.

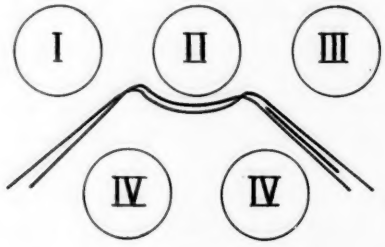

Fig. 4.—Diagram from agar plate: I. ether-extracted Rous agent; II. lipoprotein particles (after deoxycholate treatment of the agent); III. deoxycholate-treated Rous agent; IV. rabbit anti-Rous sarcoma (cytoplasm) antiserum.

Harris and Simons[68] have investigated the antigenic components of agent preparations by the agar-diffusion method of Ouchterlony.[87] Hyperimmune chicken sera produced in initially virus-resistant chicken by continued intravenous inoculation with fully infective Rous agent were used, together with rabbit anti-Rous-sarcoma cytoplasm, horse antisheep red cell (anti-Forssman antiserum) and antifowl antiserum. The antigens were prepared by treatment of Rous agent preparations with sodium deoxycholate solutions or with ether at room temperature (Andrewes and Horstmann[5]) or at $-20°$ C.

Figs. 2 to 4 show the antigen-antibody interactions as revealed by the agar plates. These may be summarized as follows:

1. Deoxycholate-treated agent suspensions give three bands with rabbit antisarcoma sera. Two of these are strong bands and are identical with those given by ether-treated agent, and with antifowl antisera.

2. Deoxycholate-treated agent gives one band with hyperimmune serum which is probably identical with that given with anti-Forssman antiserum.

3. Ether-treated agent is Forssman negative.

4. Attempts to recombine the ether-treated agent and the ether extract to reconstitute the Forssman antigen were unsuccessful.

5. The lipoprotein small granules resulting from deoxycholate disaggregation are virus antigen-negative and Forssman-negative but appear to contain 2 host antigens identical with those obtained from ether-treated agent.

6. The identity reaction is illustrated in Fig. 4. Only the deoxycholate-treated whole agent preparation reacts with anti-Forssman antiserum and gives a third band with rabbit anti-Rous cytoplasm antiserum. The other two antigens are common to the lipoprotein small granules and to ether-extracted agent.

Harris and Simons[68] concluded that the virus antigen of the agent is lipoprotein and probably related to Forssman and that the agent also contains one, and probably two, further antigens related to normal host components.

These conclusions are consistent with the data obtained by paper electrophoresis of deoxycholate-treated suspensions. The particulate material, smaller in size than the agent and deposited only at 105,000 Gm. did not migrate at pH 8.0 and stained with both azocarmine and with Sudan black, i.e., it was lipoprotein. The soluble material gave three bands. The first, which migrated toward the anode was protein-free and contained pentosenucleic acid. The fastest band migrating to the cathode was lipid in character and contained both cholesterol and plasmal. A more slowly migrating positively charged band consisted of protein only.

It may be argued that preparations of "normal" microsomes, e.g., from adult chicken liver, will behave in the same way. Physicochemically, i.e., in their rate of disaggregation by sodium deoxycholate, this would appear to be so. However, no "virus" antigen is released, and the properties of the small particles liberated are being compared with those liberated from Rous agent preparations. If the structures seen in electron-micrographs do indeed refer to Rous virus, then it is possible to draw together the "leads" obtained from infectivity and biochemical studies and to suggest the following hypothetical structure (Fig. 5).

	CHEMISTRY	EVIDENCE
Membrane I	Lipoprotein	Osmiophilic in e.m., lipoprotein, virus antigen, Forssman-like
Membrane II Ground substance I-II	Lipoprotein, mucoprotein, nucleoprotein (?)	Disaggregation experiments
"Core" III	Lipoprotein of host origin	Host-protein antigens but not "virus"

It is unlikely that nucleic acid or nucleoproteins are present in the outer membrane, since incubation with ribonuclease or with trypsin has no effect on

infectivity. The suggestion that membrane II and the ground substance between the core and membrane I contain nucleoprotein and mucoprotein derives from the rapid loss of infectivity in the presence of sodium deoxycholate and sodium borate which might form a complex with the sugar components and thus increase the rate of breakdown of the agent, and the production of low molecular weight, diffusible nucleic acid components, in disaggregation.

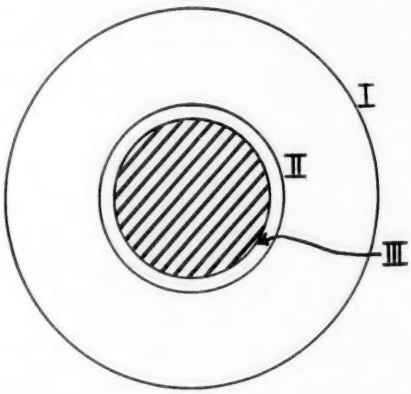

Fig. 5.—Hypothetical structure for Rous agent. (Diameter I, 70-80 mμ; II, 40-45 mμ; III, 30-40 mμ)

Methods are now being investigated for taking the agent to pieces in such a way that larger fragments, especially of the "core," are produced.

The evidence from immunological investigations of the intact agent, with respect to infectivity for the chick may be summarized:

1. One hundred per cent inhibition by hyperimmune chicken serum (10^7 minimal infective doses neutralized by the serum at a dilution of 1:100).

2. The rabbit anti-Rous cytoplasm antiserum gave a lesser inhibition (c. 50 per cent). Complement was without effect on this neutralization.

3. Antifowl antisera and horse anti-Forssman antisera were without effect, in the presence or absence of complement.

Contributory evidence as to the part played by the Forssman-like antigen has been obtained by the study of acquired tolerance of turkeys to Rous agent.[65] This will be considered in detail below, but it will suffice to state now that, as a result of the study, we have confidence that the Forssman-like "virus" antigen is related directly to the infectivity of the virus and may, therefore, be considered to be integral with it.

A similar conclusion has been reached by Beard and his associates[8] for avian myeloblastosis virus which was neutralized by anti-Forssman antiserum. On the other hand avian erythroblastosis virus was not so neutralized.

Beard postulates three components for myeloblastosis: virus specific, normal host, and Forssman. We are unable to distinguish a separate virus *and* Forssman antigen for Rous agent and conclude that this agent has a Forssman-like virus antigen and at least one host protein antigen.

ASSAY AND MULTIPLICATION OF AVIAN TUMOR AGENTS

Carr and Harris[27] reported completely successful assays of Rous agent by means of tumor-incidence response and the serial-dilution method usually employed for the assay of other animal viruses. Kenzy[72] has used the same method. In a most comprehensive and useful study Bryan[19] has evaluated the procedures to be adopted when the test chicken population is heterogeneous with respect to tumor-incidence response, and where only one inoculation site is used in each test bird.

Whereas Carr and Harris used 1-day-old chicks, (i.e., at maximum virus susceptibility), from a Brown Leghorn strain maintained for maximum sensitivity, Bryan had recourse only to New Hampshire Red of commercial origin and used them at 23 days old. The statistical method employed by him involved treating the latent period time-frequency curves as truncated normal distributions. This takes care of variability in total susceptibility, especially at high virus dilutions, and also of tumor regression at such dilutions. It has been observed before that in some chicken strains 1-day-old chicks are more resistant to infection than those which are several weeks old. Andrewes[4] first obtained evidence that the yolk of eggs from virus-immune hens contained a substance that neutralized Rous agent and this has been confirmed by Bang[6] who further found that this passively transferred antibody disappeared from the serum within the first few weeks. Bang also found that, of 8 6-month-old fowls, 4 had antibodies in serum initially, but 3 of these lost them within 6 months. We have observed a similar phenomenon in birds initially resistant to virus inoculation[65]; in these cases, too, the antibody level began to drop and tumors then began to appear at the site of the original inoculation. This is at variance with Rubin's contention that only antifowl antibodies inhibit the growth of Rous sarcoma cells.[92]

There are two other assay systems for Rous agent: (1) the production of hemorrhagic lesions in the chick embryo, and (2) the production of ectodermal lesions on the chorioallantoic membrane of the embryonated egg.

Lo and Bang[81] found that the appearance of the hemorrhagic lesions in endothelial cells of the embryo, first studied systematically by Milford and Duran-Reynals [84] was favored by increasing the incubator temperature to 39° C., by intravenous inoculation of the agent and by the use of 8- to 10-day-old embryos. Whether or not a "tumor" was produced in the membrane depended on the age of the embryo. None inoculated on the third day of incubation showed any sign of "tumor," the tenth day was the optimum period, and after 12 days few, if any, showed any lesions.

Application of this response of young embryos to virus assay showed[82] that the sensitivity was much lower than that of tumor production in the chick but was, nevertheless, adequate for the comparative studies undertaken by these authors. Assays reported by Borsos and Bang[17] involved the counting of hemorrhagic lesions on all extraembryonic membranes as well as in the embryo itself. At the end-point (log-5) each of 3 eggs out of a total of 16 were recorded as having 1 lesion each; at log-4, out of 15, 1 had 6, 2 had 5, 1 had 4, 2 had 3, 1 had 2, 3

had only 1, and 5 had none. It is thus obvious that such an assay method needs great experience and great care.

The pock-counting method for tumor virus assay was introduced by Keogh[73] and has since been used by Rubin[91,92] and by Groupé, Dunkel, and Manaker.[60] The results obtained by Rubin for the rate of multiplication of the virus will be considered below. So far as the assay method is concerned, we may observe that 12-day-old embryos were used (Lo and Bang[81]) and that they were incubated for one day in a "dry" incubator at 38° C. to evaporate the inoculum (0.05 ml.) quickly and to "reduce the danger of re-infection." Since there is no evidence that "new" virus is produced in such a short period (Harris[63]) this would seem to be the wrong reason for a procedure which may have been justified on other grounds, e.g., a decrease in the number of membranes with confluent peripheral tumors. Rubin claimed that counts of tumors on groups of membranes having an average of 100 to 300 tumors had the lowest standard error—stated elsewhere[93] to be of the order of 30 to 50 per cent. However, infrequent membranes having no tumors in such groups were discarded, and considerable variation occurred when low concentrations of virus were used. These facts suggest that there was an appreciable variation in sensitivity among different embryos. The titers agreed with the *average* of I. D. 50 assays in chicken by Bryan but it would not appear that there is any justification for the assumption, from such data, that one virus particle is adequate to initiate tumor development. Groupé and associates[60] claim to have improved the pock-counting method by using younger (9-day-old) embryos, incubating the eggs for 10 days at 38° C., using great care in making the artificial air-space, diluting the virus suspension in an improved medium, and finally, and probably most important, returning the air-space to its normal position after inoculation by stacking the eggs vertically. Despite such improvements, the sensitivity of this assay appears to be no greater than that of the tumor-incidence assay in young chicks. It has the considerable advantage, however, of ease and speed.

Four different systems have been used in studies of the multiplication of Rous virus. Carr[26] inoculated the virus into the leg muscles of 1-day-old chicks, removed the muscles after different time intervals, extracted the virus, and assayed it in the same system. Harris[63] infected the chorioallantoic membrane, subsequently extracted the virus from the membranes, and assayed it in 1-day-old chicks. Rubin[91] used single cell cultures of the sarcoma(produced by trypsin treatment) suspended in a horse serum medium. The cells in these cultures did not multiply, and the liberated virus was assayed by the pock-counting method. Groupé and Rauscher[58] propagated the virus in chick brain[61] and assayed it, after extraction from the infected brain tissue, in the wing web of very young chicks.

Rubin's results may be considered first because he was only concerned with the production of virus by tumor cells, and not with the infective process itself. Under constant conditions the sarcoma cell suspensions released about one tumor-forming particle (t.-f.p.) (equated by Rubin with one virus particle) per 100 cells per hour. When disrupted by freezing and thawing, the washed cells contained an average of one t.-f.p. for every 230 cells. Rubin suggested that all

the sarcoma cells produce virus very slowly during one generation time and that all the cells survive. Carr[26] and Groupé and Rauscher[58] both concluded that the growth curve of Rous virus parallels that of other animal viruses. There are three phases: (a) a large proportion of the infective virus rapidly disappears from the tissue; (b) there is a latent or stationary phase during which the infective-virus content of the tissue remains low; and (c) a progressive increase in virus concentration in the tissue. In chick brain the stationary phase lasted about 72 hours during which the infective virus content of the brain remained low. From 96 hours onward, however, the virus content of tissue increased logarithmically and reached its maximum in moribund chicks. Harris[63] found that virus was taken up by the chorioallantoic membrane over a period of 3 hours, for after this time the addition of antivirus antiserum had no neutralizing action. In the first 24 hours only about 1 per cent of the infectivity of the inoculum could be recovered. By 48 hours, however, the full amount was present. In the next 24 hours, rapid synthesis of infective virus occurred, parallel with the development of ectodermal lesions in the membrane. Thereafter, the high rate of virus production was not maintained. The ectodermal lesions became confluent and mesodermal tumors appeared. It was significant, too, that the acid phosphatase activity of the membrane rose by 80 per cent in the period 24 to 48 hours, for this enzyme has been shown to be associated with Rous virus.[62]

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Rubin[91] states that the virus has the capacity for causing two distinct types of tumor—ectodermal and mesodermal—depending on the cell type exposed to the virus. Histologic examination of infected membranes does not suggest to us that the ectodermal lesion should properly be called a tumor. Transplantation of isolated ectodermal lesions into the leg muscle of chicks, or back on to the chorioallantoic membrane leads to the production of the usual sarcoma only. The mesodermal proliferation at the base of each ectodermal lesion—which may occur much earlier than the 8 or 9 days quoted by Rubin—may be the result of infection of the mesoderm by the virus produced and liberated by the infected epithelium, or of invasion of the mesoderm by these infected cells. We have also observed occasional endodermal lesions, and it is well known that islands of epithelium ("epithelial pearls") may be produced in the mesoderm even by nonspecific injury to the chorioallantoic membrane. Moreover, the Rous virus produces quite different, hemorrhagic lesions in chick embryos. Before these, too, are designated "tumors" it would be well to recall the observations of Foulds[41] on this subject and then to reject finally the suggestion[93] that the capacity of the virus to infect cells which *look* different "departs from the commonly accepted concept of the rigid tissue specificity of tumor virus."

The multiplication of two other avian tumor viruses has also been studied in the chorioallantoic membrane—chick system.[64] The Fujinami agent has a slower multiplication cycle than Rous agent. At 24 hours 10 per cent of the inoculum may still be recovered from the membrane. By 72 hours the total infectivity of the original inoculum may be recovered, and between 72 and 96 hours a tenfold increase in virus titer occurs. The period of rapid virus increase is again paralleled by macroscopic changes in the membrane, and by a 50 to 100 per cent increase in specific acid phosphatase between 24 and 96 hours. The

cycle for the M.H.2 endothelioma virus has features in common with both Rous and Fujinami viruses. Absorption seems to be as rapid as for the former (less than 1 per cent recoverable after 24 hours) but the rate of multiplication is similar to the latter. One hundred per cent of the inoculum is not recoverable before 72-96 hours when the endothelial lesions become apparent in the membranes. Thereafter the virus titer increases slowly and, even by 144 hours, may not have reached one hundred times the titer of the inoculum.

The typical lesions produced by Fujinami virus are shown in Fig. 6.

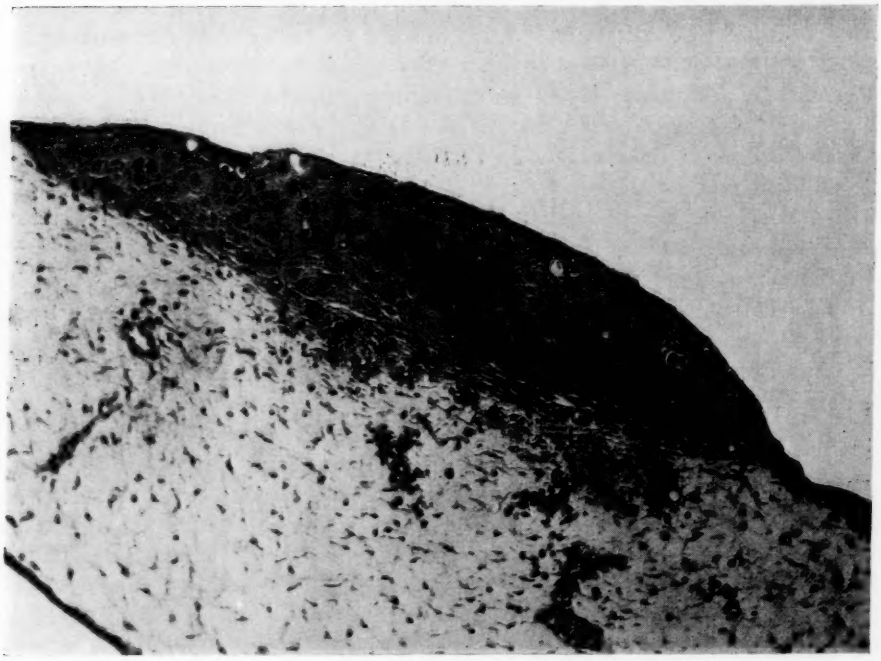

Fig. 6.—Ectodermal lesion produced on the chorioallantoic membrane by Fujinami virus.

"NONVIRAL" TUMORS

It has long been known that the tumor-producing activity of Rous agent prepared from different tumors could show extreme variability. This has been variously attributed to (a) the age of the host chicken in which the tumor tissue is growing, and (b) the age of the tumor itself, i.e., the length of time that it has grown in its host. Bryan, Calnan, and Moloney[21] found that weak inocula, containing only one 50 per cent effective dose (corresponding to a dilution of standard virus of between 10^{-5} and 10^{-6}), produced tumors from which relatively little or no virus could be recovered by extraction. Four out of seventeen such "low-dose" tumors yielded no demonstrable virus under "optimum technica

conditions." These authors concluded that the amount of extractable virus is primarily related to the initiating dose, rather than secondarily through influence on the time of development and growth, which is also related to dose.

In view of the importance of this finding we have attempted to repeat these observations but, in our chicks, five serial passages of virus using each time, as the virus source, only tumors produced by the *weakest* inocula, have failed to confirm it. After the five passages the virus titer was as high as it was at the beginning (2×10^{-6} to 2×10^{-7} minimal infective doses per gram of tumor).

We conclude, therefore, that a third factor (c) is the heterogeneity of the hosts with respect to natural resistance to the virus. This factor will be most important when the inocula are weak. It is almost certainly the one which is operating in these cases and, indeed, the factor which complicates life so much for Bryan and his colleagues. Harris[65] has shown that a very slow-growing tumor arising late in the life of one old fowl as the result of a decreasing host resistance, yet contained some virus (about 10 minimum infective doses per gram), and the tumors produced by this virus themselves contained the normal amount. Calnan, Kvedar, and Bryan[25] record their failure to produce a "virus-free" line of the tumor by serial *cell* transplantation using only the most slow-growing tumor for each successive cell passage.

A similar claim[59] to have produced "nonviral" tumors in turkeys with Rous virus will be examined in another context.

Whatever factors may be operating to reduce the virus yield of these tumors it is surely carrying conviction too far to suggest that these phenomena cast light on the "nonfilterability" of most mammalian and avian tumors. Unless and until an infective subcellular agent has been *demonstrated* to be the cause of a particular tumor we shall continue to regard that tumor as not caused by a virus. It is perverse logic to suggest that failure to demonstrate virus in filtrates of tumor-tissue extracts is *not* justification for their classification as nonvirus tumors.

ACQUIRED TOLERANCE AND TUMOR VIRUSES

Burnet and Fenner[23] postulated that any potentially antigenic "pattern" constantly present during embryonic life could permanently condition the scavenging cells against immunologic response to this particular pattern. Burnet, Stone, and Edney[24] attempted an experimental investigation of this by injecting antigens into chick embryos by appropriate routes, allowing the chick to hatch and, when the bird was 5 to 8 weeks old, first testing its serum for antibody to the same antigen before establishing the bird's capacity to respond to an immunizing inoculation of it. The most convincing demonstration of the hypothesis had been provided by Traub[108] who described a mouse colony in which almost all the individuals were infected with the virus of lymphocytic choriomeningitis in utero. If they were born alive they carried through life a heavy, generalized infection with the virus without, however, either showing symptoms or producing antibody. Burnet and his colleagues failed to show any change in the subsequent response of the hatched chicks to influenza A virus, bacterial virus C16, or washed human red cells following treatment of the embryos with these antigens.

Billingham, Brent, and Medawar,[14] however, found that injection of chicks at hatching with adult chicken red cells resulted in a marked depression of the antibody response to a later injection of the homologous material. The same authors[13] had previously shown that injection of *living* cell suspensions into embryos, either of mouse or of chicken, produced a state of immunological tolerance such that the injected individuals would accept skin grafts, in postnatal life, from the original donors of the cells. The antibody depressions were not restricted to intraspecies combinations for Hasek[69] observed that the injection of goose blood into duck embryos resulted in an obvious suppression of later antibody formation, although no such suppression was observed when turkey blood was injected into chick embryos, guinea fowl into chick, duck into chick, chick into duck, duck into goose, or chick into guinea fowl. Simonsen[97] found suppression of agglutinin formation in turkeys injected as embryos with adult chicken blood and vice versa. Tolerance was not so readily acquired between goose and chicken. Harris and Simonsen[64,65,97,98] also found that this tolerance between chicken and turkey extended to infection with Rous virus. American Mammoth Bronze turkeys more than a few weeks old are resistant to Rous agent infection. If, however, they have been injected, as embryos or as 1-day-old poults, with small quantities of adult chicken blood they retain a sensitivity to the agent and respond by the production of sarcomas at the site of inoculation.

In the tissue transplantation studies of Medawar and his colleagues it became apparent that the foreign living cells introduced into the embryo were accepted as transplants and conferred a persistent tolerance *only* toward tissues of the same antigenic specificity as the donor. Moreover, tissue transplantation immunity seemed to depend in large part on a different mode of immune response than the development of circulating serum antibody. It appeared to involve antigens that were distinct from those associated with erythrocytes since the depression in the development of circulating antibodies against erythrocytes, induced by injecting pure suspensions of red cells into chick embryos was not associated with transplantation tolerance.[14] The capacity of whole blood to confer tolerance to tissue transplantation resides entirely in the white cells, and the antigens responsible appear to be those of the cell nucleus in contrast with the cytoplasmic antigens of the serum antibody system.[15] Antigens of spleen cell nuclei have been shown to confer transplantation immunity when injected into immunologically mature animals,[16] but transplantation tolerance has, as yet, only been conferred upon embryos or young animals in the presence of intact donor cells.

In the investigation of the acquired tolerance of turkeys to Rous sarcoma virus, there were two major questions which required answers. First, was the tolerance of the tissue transplantation or of the serum antibody type, and second, what, if any, was the relationship of the antigen(s) responsible for conferring tolerance to the antigens of the infective virus? Young turkeys may be infected by the virus, and it appeared probable, therefore, that the resistance of older birds to the virus was a result of a natural or acquired immunity of the serum antibody type.

It was quickly found that it was not necessary to use turkey embryos because 1-day-old poults could equally well be made tolerant to Rous virus. At

3 days old, however, the immunologically plastic period had passed.[65] The antigens responsible for conferring tolerance were present only in the cellular fraction of whole fowl blood, and they withstood freeze-drying without impairment. Moreover, they were not completely destroyed by heating (49° C., 20 minutes) sufficient to abolish their tolerance-conferring property both for skin homografts and, in the case of turkeys and chickens, for skin heterografts.[12]

The property of conferring tolerance was soon found to be nonspecific. Blood from a different strain of fowls was fully competent, and turkey poults given pigeon blood (and a pigeon skin graft) on the day of hatching promptly destroyed the pigeon skin but retained a high sensitivity to the tumor virus. Similarly guinea pig blood cells formolized sheep cells and chicken spleen cell suspensions,[66] conferred tolerance, but rat blood cells did not; human Group A (Rh +) conferred tolerance but Group O did not. These data gave support to the idea that Forssman-type antigens should be investigated. They have been postulated as components of fowl sarcoma viruses[46] and have recently been shown to be closely associated with fowl myeloblastosis virus.[8]

So far as the sarcoma viruses are concerned, their presence could not be held to be unequivocal evidence of their association with the viruses themselves for the cytoplasmic granules of normal chicken tissues are also Forssman-positive.

The tolerance-conferring antigens of chicken blood were found to be stable to freeze-drying, and attempts were then made to isolate a soluble antigen from the blood cells. Phenomena comparable with tissue transplantation tolerance had been produced with soluble antigens, especially in rabbits by Dixon and Maurer,[33] Cinader and Dubert,[28,29] Smith and Bridges,[99] and Downe[37]; but also in chicken by Wolfe, Tempelis, Mueller, and Reibel,[111] and Hasek.[69]

Carefully prepared "ghosts" from chicken red blood cells conferred tolerance and could be safely administered intravenously. Crude stromal preparations were inactive. Tolerance could also be conferred by intraperitoneal rather than by intravenous inoculation of whole cells, although the response was reduced. Intraperitoneal injection gave a 43 per cent tumor response in the treated turkeys compared with 61 per cent by the intravenous route. It was necessary to establish the extent of this difference because it proved impossible to give extracts of the blood cells by the intravenous route. The extract was prepared by the method of Kirby[74] and contained deoxypentose- and pentosenucleic acids, protein and other components, probably muco- and lipoprotein in nature. The tumor response, following treatment of 1-day-old poults with the extract at an equivalent concentration to whole blood, was comparable with that obtained by the use of whole washed cells. Medawar and his colleagues have demonstrated that transplantation *immunity* may be transferred in mice, not only with intact spleen cells but also with an extract of spleen cell nuclei, containing predominantly desoxypentosenucleic acid.[16] Treatment of the chicken blood cell extract with trypsin and chymotrypsin, with deoxyribonuclease or ribonuclease, or with both nucleases had no significant effect on its tolerance-conferring property. Moreover, the residue after combined nuclease degradation contained the sugar fucose which is a component of the blood group substances.

Experiments to confirm that blood Group A substances are indeed responsible for these phenomena are in progress. Its heterogenetic character, stability to heat and to enzymic degradation, activity at very low concentrations (0.3 ml. chicken blood cells must contain very little), and differentiation from the tissue-transplantation antigens and, so far as Group A substance is concerned, its Forssman-like activity, make it probable that the antigen is either blood Group A substance or something very similar. For example, it inhibits at very low concentrations the agglutination of A, Rh positive human erythrocytes by anti-A antiserum.

The assumption has been made that the antigens responsible for conferring tolerance were identical with those comprising the Rous virus itself. This assumption appeared warranted in the grounds of the apparent specificity of acquired immunological tolerance. We have been unable, however, to extract significant amounts of such antigen (s) from Rous agent itself. The degree of tolerance produced by extracts of the agent was not significantly different from the control level. This may, however, be purely a question of preparing enough of the material now that we have a clearer idea of what to look for.

Harris[65] showed that turkeys could also be made tolerant to grafts of the nonfilterable, carcinogen-induced chicken sarcoma G.R.C.H. 15. This tolerance was produced by the use of whole chicken blood, and it will be interesting to see if soluble extracts of these cells can confer tolerance to grafts or whether, as one might expect, this will prove to be of the tissue transplantation type, requiring intact, living leukocytes.

The production of tolerance with soluble antigens in this way enables us to refute Svoboda and Hasek,[107] who, having confirmed that tolerance to Rous sarcoma could be produced in turkeys by intravenous inoculation of whole adult chicken blood, suggested that the hen blood elements, particularly the monocytes, may have survived, may have been infected by the injected virus, and may have continued to act as a cellular inoculum which always gives a positive result on transplantation.

Similarly, their further suggestion that the hen blood may have contained subinfective quantities of intact Rous virus, or of an immunologically indistinguishable virus, is ruled out because the method used to prepare the soluble, cell-free extract would destroy *intact* virus. Svoboda and Hasek used a crude extract of fresh Rous sarcoma which, at a tissue concentration of 20 per cent, and in the absence of hyaluronidase was only centrifuged twice at 3,000 r.p.m. in siliconed test tubes. It is possible, therefore, that their extract was *not* cell-free.

These authors also found normal formation of immune agglutinins to hen red cells in one series and postulated that the fowl (host) antigenic component of the virus does not correspond to the erythrocyte antigen but to the antigens of the tissue cells responsible for transplantation immunity to tissue grafts. In view of the heterogenetic nature of the phenomenon, this suggestion, too, is improbable.

It has been shown, however,[66] that tumor-bearing turkeys which have been made tolerant to Rous virus, fail to produce antibodies to the virus, i.e., anti-

virus antibodies which neutralize the infectivity of the virus for the natural, chick host. The results are shown in Table I.

The division of the tolerant turkeys into the three groups is arbitrary but separates them into the slightly tolerant, more tolerant, and very tolerant. The percentages refer to the proportion of birds in the whole group which developed tumors; the sera tested, except for the controls, were from the tumor-bearing birds.

This demonstrates that, whatever the mechanism by which tolerance is conferred, it is associated with failure of the turkey to produce, not necessarily hemagglutinins for chicken red cells[107] but virus-inhibiting antibodies.

It is worth noting, here, that inhibitory sera were so designated only when *no* tumors appeared in the test chicks, i.e., the virus was fully neutralized by 1:10 serum dilutions in the absence of complement. Rubin[92] has studied the apparent neutralization of Rous sarcoma virus with antiserum to normal chick embryo (actually stated to be normal chick). The results obtained show that only very weak serum (and weak antiviral antiserum, too) was available, the neutralization rarely exceeding 10^1 or 10^2 compared with 10^3 to 10^4 obtained by us. Moreover the results of single experiments only appear to be quoted. In the *one* experiment, for example, in which rabbit antichick embryo serum was added at 1 hour, 4 hours, or 48 hours after infection of the chorioallantoic membrane with Rous virus the results are given with no data for the effect of adding normal rabbit serum at 4 or 48 hours. These incomplete experiments can hardly be said to support adequately the conclusion that antichick serum inhibits tumor formation by the cells of the chorioallantoic membrane by damaging the cells rather than by neutralizing the virus.

TABLE I.

TURKEYS	NUMBER	SERA		
		NONINHIBITORY −	±	INHIBITORY +
Control	16	16	0	0
Tolerant (5 to 25%)	6	1	1	4
Tolerant (25 to 50%)	19	6	6	7
Tolerant (50 to 100%)	12	7	3	2

The small drop in infectivity produced by the action of antichick antiserum and its reversibility on dilution can be, and have been, explained by the adsorption of virus to nonviral chicken antigen-antibody aggregates. It would also be expected (and this Rubin found) that antivirus antiserum was not absorbed by chick embryo cells whereas the antichick serum was.

PROPERTIES OF TUMORS INDUCED IN TOLERANT TURKEYS

One of the virus-induced turkey tumors was transplanted serially, by cell graft, in very young turkeys for over thirty generations. Throughout this

period the tumor (Fig. 7) remained uniform in rate of growth and general pathology. It was extremely malignant and killed the young host within about 10 days with widespread metastatic deposits (Figs. 8 and 9), a particularly noticeable feature being a virtual exsanguination from hemorrhagic lesions of the spleen or liver. Andrewes[3] has described similar lesions in tumor-bearing pheasants; it may also be no coincidence that pheasants are apparently in the same Forssman group as chicken and Duran-Reynals found them in tumor-bearing turkeys and guinea fowl. The viruses extracted by Duran-Reynals from his turkey and guinea fowl tumors regularly infected adult chicken and showed, in such hosts, the same tissue affinities as in the respective original hosts. This difference from the normal behavior of the Rous agent in chicken was attributed by Duran-Reynals to a variation in the tissue affinity of the agent.

Fig. 7.—Turkey sarcoma produced by Rous agent. Tenth transplant generation.

Although our turkey tumor was apparently behaving uniformly during serial transplantation, changes were found to have occurred in the infectivity for chicks of the agent derived from the tumors. The virus was extracted from the tumors in the usual way, separated from cellular material by differential centrifugation, and assayed in 1-day-old chicks by the procedure described by Carr and Harris.[27]

Table II shows that in twenty generations the infectivity for *chicks* had dropped from 2×10^5 minimum infected doses per gram to 2×10^2. However,

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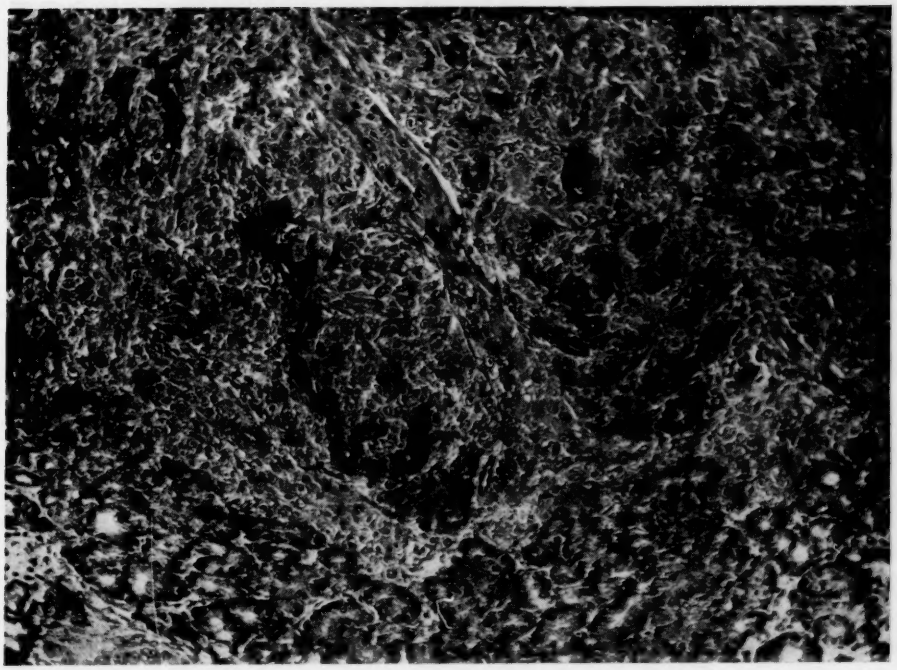

Fig. 8.—Turkey sarcoma produced by Rous agent. Metastasis in pancreas. Tenth transplant generation.

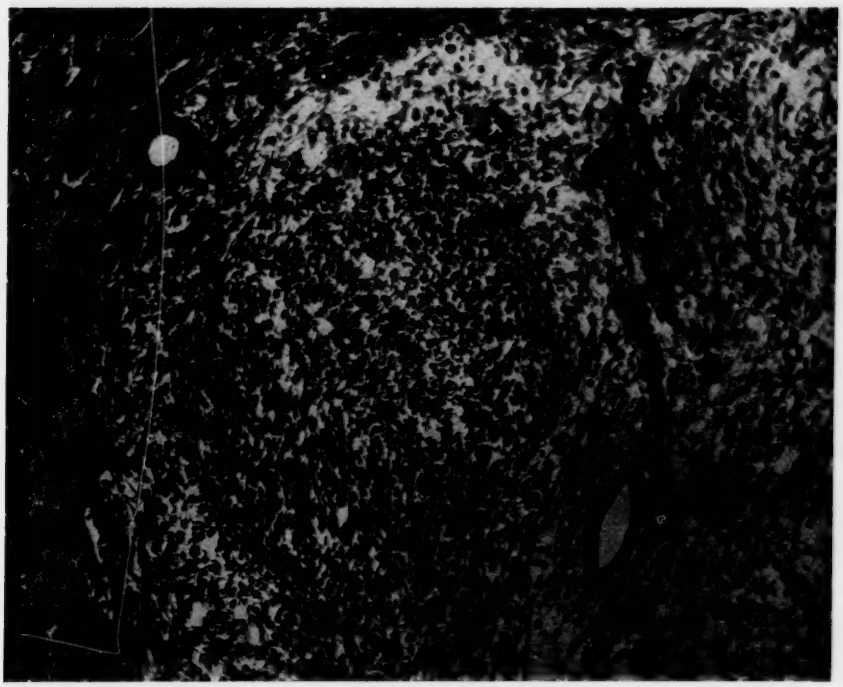

Fig. 9.—Turkey sarcoma produced by Rous agent. Metastasis in liver. Tenth transplant generation.

the agent content of the *chicken* tumor produced by this "turkey" virus was at least as great (2×10^6 minimum infective doses per gram) as that of normal Rous sarcoma propagated solely in chickens.

The infectivity for *turkeys* was 2×10^6 minimum infective doses per gram in the later generations of the turkey tumor. The extent of this variation was such that we hoped to be able to produce a turkey tumor containing virus fully infective for turkeys but noninfective for chicken. We have not been able to do this.

Groupé and Rauscher[59] found that serial passage of Rous sarcoma *virus* in 3- to 6-day turkey poults resulted in complete loss of virus after the fourth passage and, moreover, that turkey sarcomas produced with as much as 10,000 I. D. 50 of chicken tumor virus yielded little or no extractable virus as assayed in chicken. We cannot confirm these results.

TABLE II.

GENERATION NUMBER	MINIMUM INFECTIVE DOSES PER GRAM TUMOR
3	2×10^5
10	2×10^4
23	2×10^2

VIRUS-INDUCED TUMORS IN MICE

Until the recent discovery of leukemia-producing agents, milk factor was the only known virus with tumor-producing activity for mice. There is still some dispute as to the nature of the leukemic agents, and they are not universally accepted as virus (*vide infra*). Milk factor, itself, differs from the avian tumor viruses in its mode of action. It can exist in a latent form in the organs of both male and female mice of the affected strain, having been acquired from the mother's milk, and it can apparently remain in the body for a long period without producing tumors. A chemical initiating cause—estrogen—activates it in the genetically susceptible female in the course of 7 to 12 months and mammary cancer develops. The males do not normally develop cancer but tumors can be produced by the administration of estrogens. The milk factor will also produce tumors after intraperitoneal inoculation into susceptible mice. Below a threshold dose an appreciable number of the animals may fail to develop tumors themselves, but their first and second generation descendants may show an increased tumor incidence.[1] Particles isolated from mammary tumors, and held to be the milk factor, show antigenic differences from similar particles isolated from normal mammary tissue, but are themselves very weak antigens in the mouse.[36] Both high and low cancer line mice seem devoid of serum antibodies to milk factor.

There is general agreement, too, that milk factor should be given in the first few days of the life of the mouse. Adults are much more resistant to the virus, although this resistance can sometimes be broken down by repeated injection or by a single massive dose of virus. The properties of the milk factor have been reviewed at great length recently,[34] and we shall not attempt to summarize this

here. It will suffice to note from all these data three points for further discussion: (1) the necessity for introducing the milk factor into very young animals; (2) its weak antigenicity; and (3) the long latent period between the introduction of the virus and the production of a tumor.

There are two groups of leukemia-producing viruses for mice. The first group of viruses has been derived from mesenchymal tumors such as the Ehrlich carcinoma. The virus isolated by Friend[44,45] was derived from the spleen of a 14-month-old Swiss mouse inoculated in infancy with cell-free extract of Ehrlich tumor cells. The mouse showed no evidence of a tumor, but the spleen and liver were grossly enlarged and leukemic. The leukemia was serially passaged 3 times with spleen suspensions before the first cell-free passage was attempted. After inoculation of the virus (which has an L. $D_{.50}$ in filtrates of about $10^{-3.2}$, and passes a "Gradocol" membrane of a.p.d. 220 mμ) there is a latent period of about two weeks, after which the spleens become enlarged and palpable. About 85 per cent of the infected mice die, and the media survival time was 77 days. Swiss and DBA/2 strain mice alone were susceptible. PR1, C57B1./6, A, C_3H and C58 × BALB were resistant.

The virus studied by Graffi and his colleagues[11,51-54] was derived from a variety of mouse tumors—Landschutz sarcomas I and II, Ehrlich carcinoma (in both solid and ascites forms), SOV-16 (a myeloid leukemia which arose following injection of a cell-free filtrate from sarcoma I), and sarcoma 37. Suckling Agnes-Bluhm strain mice were injected subcutaneously at the age of 1 to 3 days. Adult mice could also be infected, but the tumor (chloroleukemia) incidence was reduced. Using newborn mice the chloroleukemia incidence was high (more than 50 per cent from any source of virus), and the average latent period varied between 124 and 320 days. In adult mice, the incidence was less than 30 per cent, but the latent period was about the same. The highest incidence of *spontaneous* leukemia in the strains of mice used for virus assay was stated to be 1.3 per cent, but one perturbing feature of this work was the poor transferability of the induced chloroleukemias by cell graft. This suggests either that the tumors were not malignant or that the strains of mice were unduly heterogeneous. Newborn rats could not be infected, and filtrates from mouse, rat, or chicken embryo gave a 5.5 per cent incidence of leukemia. There was no chloroleukemia.

The production of leukemia in mice after injection of normal tissue filtrates has also been studied by Schmidt and Lohmann[94] using the same strains of mice as Graffi and associates.[11,51-54] Mouse embryo filtrate gave 7 per cent leukemia or lymphosarcoma in females and 9 per cent in males, and rat embryo filtrate 3 per cent in females and 4 per cent in males. The spontaneous incidence was 2 per cent. The same authors[83] produced myeloid leukemia in Agnes-Bluhm mice with cell-free filtrates from six different transplantable mouse tumors.

The properties of the tumor filtrates described by these three groups of authors are similar—but quite nonspecific. All three record the destruction of the activity after incubating for 30 minutes at 56° C. The filtrates appear to be stable between pH 4.0 and pH 9.5. Graffi and his colleagues showed that a "nucleoprotein" precipitate from the filtrate was leukemia-producing and that the activity was not destroyed by incubation with ribonuclease or deoxyribonuclease.[11,55]

Immunization of rabbits with filtrates or sediments produced by centrifugation of the filtrates gave antisera that almost completely inhibited the leukemia-producing activity. However, a rabbit immune serum against normal mouse lymphoid tissue also had an inactivating action on sarcoma I filtrates. Attempts to produce antisera in mice were unsuccessful.[50]

In the second group are those viruses derived from spontaneous leukemias in certain susceptible strains of mice (such as AkR) and purported to be tested in young mice of a *leukemia-free* strain (such as C_3H and C_3Hf). Like the milk factor, this virus or group of viruses (for tumors other than leukemia may also be produced) have to be introduced into very young mice, usually less than 1 day old. There follows a long latent period before the tumors arise.

Gross,[57] who reviewed 7 years of experiment in 1957, used mice less than 16 hours old for the assay of cell-free extracts, prepared by filtration, or by differential centrifugation. Older suckling mice gave erratic results, and adult mice were resistant. With C57 Brown/cd as the assay strain, filtered Ak leukemia extracts gave only leukemia, C_3H and C_3Hf strains developed leukemia, parotid gland tumors, and subcutaneous fibromyxosarcomas.

The spontaneous incidence of leukemia in these mice was claimed to be 0.5 per cent but among the An, He, and Lw sublines of C_3H maintained at the National Cancer Institute, Law[78] found that the incidence could be as high as 10 per cent. Moreover, the C_3H strain transferred to Figge in 1942 (C_3H/Fg) became a high-leukemia strain in 1952, and transferred further to Law in 1953 and fostered on C_3Hf (C_3Hf/Fg), began to produce in Law's own words "a galaxy of reticular neoplasms." In about 200 untreated mice of C_3H/Fg and C_3Hf/Fg, 6 pleomorphic parotid-gland tumors were also discovered.[78]

Gross used litter-mate mice inoculated with heated filtrates (65 to 68° C. for 30 minutes) as his controls. The results are summarized in Table III.

Gross[56] also discovered that parotid gland tumors could be induced with normal organ extracts from C_3H or C57 Brown.

Physical data claimed for the "Gross" agent are ether-sensitivity, stability to freezing and a size of 50 to 70 mμ as determined from electron-microscope and ultracentrifuge data.

The leukemias could be cell-grafted into C_3H mice producing acute leukemia within 2 to 3 weeks but only rarely could they be back transplanted to Ak. The parotid gland tumors would also grow in C_3H or C_3Hf, and in a few Ak mice less than 12 hours old. Adult Ak mice were resistant. It thus appears that these could be virus-induced tumors with the immunogenetic character of the recipient (C_3H) strain rather than that of the donor (Ak) strain. Woolley and Small,[112] using the same procedures as Gross, obtained very similar results. Furth and colleagues,[47] however, reported a heterogeneity of leukemic types induced in low-leukemia strains; some were related immunogenetically to both host and donor strains. Tissue extracts from the high leukemia strain Ak greatly *increased* the incidence of leukemia in Rf, DBA/1 and C_3Heb mice and greatly *accelerated* the development of the disease in AkR mice. Neither salivary gland nor other tumors were induced by the leukemia agent studied. Acceleration of leukemia development in C_3H mice which received at birth cell-free saline extracts of

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TABLE III

FILTRATES TESTED FROM	NUMBER OF MICE (C_3H OR C_3Hf)	RESULTS					
		LEUKEMIA (%)	AVERAGE LATENT PERIOD (MONTHS)	PAROTID GLAND TUMORS (%)	AVERAGE LATENT PERIOD (MONTHS)	SUBCUTANEOUS SARCOMAS (%)	AVERAGE LATENT PERIOD (MONTHS)
Leukemic Ak	328	28	10	11	5	1	13
Heated leukemic Ak	166	1.2	17	3	—	—	—
Leukemic C_3H	175	36	7	10	4	3	10
Parotid gland tumors, C_3H	300	16	14	7	5	10	15
Subcutaneous sarcomas, C_3H	355	7	14	5	5	4	7

leukemia cells or viable leukemia cells of a "foreign" strain has been reported by Ida, Kirschbaum, and Taylor.[71] These authors also found that mice of "low leukemia" strains (such as Bittner C_3H) could develop spontaneous leukemia at advanced age.

Furth and associates[47] observed no parotid gland or subcutaneous tumors in their treated mice, but Dulaney[38] described them in C58, AkR, and the non-related C_3H mice following injection of newborn animals with cell-free leukemic extracts of AkR or Akn. In the AkR mice which did not develop parotid gland tumors the time of appearance of "spontaneous" leukemia was not decreased.

Gross found that cell-free extracts of the parotid gland tumors produced in the C_3H and C_3Hf mice gave leukemia, parotid gland tumors and subcutaneous sarcomas following inoculation into newborn C_3H or C_3Hf mice, although by cell passage only parotid gland tumors were produced. Stewart and associates,[106] however, obtained no tumors of any kind over a period of 2 years in mice treated in a similar way with cell-free inocula prepared from 20 different parotid gland tumors. In these experiments the parotid gland tumors arose in $(C3Hf \times AkR)F_1$ hybrids,[102] and newborn hybrids from the same strains were used for the assay. When homogenates from these tumors were cultured with monkey kidney tissue for one or two passages (each of 1 or 2 weeks) and the cell-free supernatants inoculated into newborn mice, multiple tumors began to appear. Mice inoculated with fluids from tissue cultures which had not received tumor cells failed to develop these tumors. All the mice which developed tumors had both parotid gland tumors and multiple proliferative renal lesions. A variety of other primary tumors was also observed. It was concluded that the neoplasm-inducing substance in the tissue culture was derived from the tumor cells, and, moreover, that the tissue culture cells (monkey kidney or chick chorioallantoic membrane) provided a medium for its replication.

Finally, Schwartz and associates[95] claim to have accelerated the appearance of leukemia in 3- to 16-week-old AkR mice by means of cell-free filtrates of brain from human beings who died of leukemia.

This presents a picture of increasing complexity. Among the skeptics, Law[78] and Furth and his colleagues[47] believe that the onus is still upon the virus "proponents" to prove their case. For example, Stewart[103] found an increase in the incidence of lymphomas in $(C_3H \times AkR)F_1$ hybrids following the introduction of filtrates from AkR, and from other, high-leukemia strain tissues. Law, Dunn, and Boyle[80] were unable to confirm this but found instead that the hybrids themselves have a normal "spontaneous" leukemia incidence of 50 to 60 per cent, with some of the tumors occurring as early as 7 months. Irradiation, or the inoculation of AkR (but not C_3H) bone marrow, caused the tumors to to appear earlier. Law stresses the role of the thymus in the development of lymphocytic tumors. Total thymectomy in most high-leukemia strains of mice almost completely overrides the potentiality for the disease. This applies equally to "spontaneous" and to induced disease, whether or not the thymus is the primary site of origin of the disease. The phenomenon has been observed in Ak, AkR, DBA, C57B , and C58 strains. Moreover, the presence of a thymus graft in thymectomized animals partially restores the potentiality but, para-

doxically, in many cases the grafted thymus tissue does not supply the cells from which the tumor arises.[79] Nothing is known of the relationship of the tumor-inducing agent to the type of leukemia induced. In the Ak strain most spontaneous leukemias are thymic in origin and in Furth's study all the leukemias in agent-injected Ak mice were lymphoid with thymic involvement. In other strains, however, many were generalized in the presence of involuted thymuses. Does it follow that leukemia induction by Ak agent would be prevented by thymectomy? Furth[47] discusses three possible processes for the development of leukemias following injection of tissue extracts from high-leukemia strain mice: *transplantation*, *induction*, and *transduction*. The first is really a problem of technique, and it is probable that the extracts used have been cell-free. It is almost impossible, of course, to guarantee that all inocula are cell-free and equally, that all the strains used were homozygous. Gross and Woolley believe that *induction* is the process and that their tumors were characteristic of the recipient and not of the donor. The results obtained by Furth and associates[47] at least suggested that some of the tumors they induced had the genetic character of both donor and recipient. This finding leads them to suggest that the transmitting agent is related to the chromosomal nucleoprotein of the leukemic cells, or their precursors which, like the desoxypentosenucleic acid (D.N.A.) of certain microorganisms, can enter the genetic apparatus of other cells, either multiplying with them or causing perpetuation of a special genetic characteristic by some other method. This is the phenomenon of *transduction*. While admitting that their evidence is equivocal, Furth believes that a theory based on pneumococcal transformation best explains the following observed facts; the necessity for using newborn mice; the high sensitivity of strains which are genetically related to the Ak strain; the apparent noninfectious nature of the agent under natural conditions; and the very long latent period, which is about equal to that of the spontaneous disease.

Another group of experiments which has been interpreted as "transduction" is that described by Stasney, Paschkis, and Cantarow[100,101] and by Paschkis, Cantarow, and Stasney.[89] Subcutaneous injection of cell-free chromatin prepared from the Murphy rat lymphosarcoma gave 33 per cent tumor takes in young rats within two weeks. In contrast with the lymphosarcoma chromatin, hepatoma material never induced tumors when injected subcutaneously. To be effective, this had to be introduced directly into hepatic tissue. There was considerable evidence, both direct and indirect, for the absence of intact cells, but no suggestion as to how this "malignant" chromatin could be taken up by the homologous host cells and incorporated into the chromosomes.

The identification of the leukemia agents with such transducing factors (D.N.A.-containing chromatin) presents a number of biochemical difficulties. The pneumococcal transforming factor is allegedly protein-free D.N.A. The published stability data for one of the leukemia agents (Graffi) show, however, that it is heat-sensitive, ether-labile, particulate, stable to freezing, unaffected by deoxyribonuclease, and antigenic in rabbits (albeit not in mice).

It has already been suggested[65] that the mouse leukemia agent is not the direct causative agent of the disease but an antigen-bearing particle capable of interfering specifically with the immunity-producing system in the mice, since

it must be introduced into very young, immunologically plastic animals, so that other, possibly intrinsic, tumor-producing agencies are subsequently liberated.

This hypothesis, too, is relevant to the question of whether or not intact donor cells are present in the "cell-free" tissue extracts injected into the very young mice. Furth and associates have already commented on the fact, that with Ak extract, some of the leukemias produced did not behave as Ak tumors but were transplantable to both donor and recipient strains.

Such ambivalence might be explained if some of the newborn non-Ak hosts had been made tolerant to Ak cells. The leukemias arising in such animals could show both host and donor properties since Koprowski[75,76] has shown that mice made tolerant during fetal life would succumb in adult life to otherwise incompatible lymphomas. Moreover, after a single passage through *conditioned* mice, subsequent serial transfer in *untreated* individuals of the foreign strain became possible.

Mouse mammary cancer is not produced by a single factor. Of the three known major etiologic factors (genetic susceptibility, estrogen stimulation, and the milk agent) a combination of any two will apparently realize the tumor potentialities. No one of these factors has been shown to cause, under any set of conditions, an all-or-none difference.

There is a genetic basis for susceptibility to leukemia, and other leukemogenic factors—estrogens, carcinogens, and radiation are known. Synergism has been observed with combinations of these factors at subthreshold levels and, if the leukemia agent be added to the list, it may also have been observed between the agent and carcinogens; for Schmidt and Lohmann[94] claimed that whereas normal mouse embryo filtrate gave 7 per cent leukemia or lymphosarcoma in female hosts and 9 per cent in male, addition of 9:10 dimethylbenzanthracene resulted in incidences, respectively, of 6 per cent and 20 per cent.

We suggest, therefore, that to the three possible processes of transplantation, induction, and transduction should be added a fourth, *acquired immunological tolerance.*

The pathogenesis of several diseases has been ascribed to an interaction between supposed autoantibodies and tissue antigens—e.g., acquired hemolytic anemia and Hashimoto's disease—lymphadenoid goiter.[90]

Witebsky and associates[110] have also examined chronic thyroiditis in man. Twelve patients were found whose sera contained circulating antibodies specifically directed against human thyroid gland. In experimental confirmation of this, thyroiditis was produced in rabbits, dogs, and guinea pigs immunized with extracts of glands of the same species, or even of the same individual animal. In the light of the "self-marker" hypothesis of Burnet and Fenner[23] the phenomenon of autoimmunization could be explained in at least two ways. First, that the animal had had no "experience" of the particular antigen during the immunologically plastic period, i.e., it was not recognized as "self," or, second, that during the life of the animal the antigen had changed in such a way that it was no longer recognized as "self." It should be possible to distinguish these situations experimentally by, for example, giving the embryos immunological tolerance to the normal antigen, and studying the subsequent reaction to attempted immunization with the same antigen in later life.

The first explanation is of particular interest for the investigation of mouse leukemia. If an antigen, present in the embryo but unable for one reason or another then to influence the antibody-forming mechanism, was subsequently released to the circulation it would be probable that the organism would not recognize it as "self" and would produce antibodies to it. An originally intracellular antigen which became extracellular would meet this situation. Conversely there may be the condition in which there is a continuous, aberrant "leak" of antigen into the circulation, starting in the immunologically plastic period and continuing into the adult life of the animal. This would lead to a state of autoimmunological tolerance and, if the antigen was not normally available to all such animals in this way, to a difference in their subsequent history.

It is conceivable, therefore, that the thymus-dependent spontaneous leukemias of mice are the result of the interaction of an aberrant specific antigen from the thymus in animals of the right genetic susceptibility. The induction of the disease in "low-leukemia" strains by the introduction of the antigen in the immunologically plastic period would provide an acquired tolerance to the disease—not an autoimmunological tolerance.

The end result, leukemia, would be the same. Moreover, the disease would be host-specific—the result of the abrogation of host control of the host's own cells. It follows immediately from such an hypothesis that the noncellular leukemia-inducing extracts should be capable of replacing the thymus as a source of antigen; that the normal thymus itself should possess such antigens, which might well be of nuclear origin,[16] and that no antibodies to them should be present in the sera of "high-leukemia" strain mice. Antibodies should, however, be detectable in the sera of "low-leukemia" strains but very sensitive methods, similar to those employed by Witebsky and associates may well be necessary to detect them.

This hypothesis explains why leukemias and the other, parotid gland and subcutaneous tumors, are only induced in strains in which spontaneous tumors of the same types are known. It meets the requirement that the agent should be present in some normal mouse tissues and that it should have to be introduced into very young animals. It also shows how tumors induced by cell transmission in such very young animals could apparently have immunogenetic characteristics of both donor and host.

The effect of such an acquired tolerance may not be limited to the induction of leukemia, for Furth and others have noticed that in "low-leukemia" strains in which the disease developed in extract-injected animals, and was absent in the saline-injected controls, mortality from other causes was invariably higher among the extract-injected mice, the difference being greatest in the strain in which the incidence of leukemia was highest. The presence of the agent appeared to have lowered the *general resistance* of the mice to disease, possibly by interfering with normal immunity mechanisms in the animal.

Both Koprowski[77] and Burnet[22] have recently made similar suggestions, Koprowski for leukemia agent and Burnet for milk factor. Of the latter, Burnet writes: "The general picture one receives is of a virus of very low grade that has found a way of persisting in mouse tissues analogous to that of lymphocytic

choriomeningitis virus. As such it has an opportunity to act as an effective link in a chain of abrogated controls leading to the development of mammary cancer."

It is probably just coincidence that Traub,[109] in 1941, reported a higher incidence of leukemia in a mouse colony infected with lymphocytic choriomeningitis virus than in a subline of the colony that was virus-free. In 1956, Stewart and Haas[104] recovered and identified this virus in two sublines of L1210 leukemia and some of these contaminated tumors yielded cell-free extracts with high tumor-inciting properties. Further work[106] has shown, however, that this particular virus was not the agent responsible for the tumors produced.

Further work must be directed first to a correct appraisal of the genetic factors involved in spontaneous leukemia, and second, to the nature of the leukemia agent. In the latter connection the current interest in viruses lies very much with the question of the infectivity of the nucleic acids associated with them. For tobacco mosaic virus there would seem to be general agreement that the isolated ribonucleic acid is infective, albeit at a much lower titer than the intact nucleoprotein.[43]

Moreover, virus may be reconstituted by combining the nucleic acid and protein moieties, and this recombination will also take place, in these plant viruses, with protein and nucleic acid from different strains.[42] In the latter case the nature of the disease provoked by the mixed virus preparation resembled that characteristic of the virus supplying the nucleic acid.

The extension of such work to animal viruses has not been long delayed. Colter, Bird, and Brown[30] isolated ribonucleic acid (as a solution by the phenol method of Gierer and Schramm[49]) from Ehrlich ascites tumor cells infected with Mengo encephalitis virus. The preparation was infective at a level of 0.1 per cent of the control virus and was claimed to differ from it in being protein-free, destroyed by ribonuclease, destroyed by incubation at 37° C., easily sedimented in molar saline, and not sedimented at 30,000 r.p.m. in a Spinco (Model L). The virus range has since been extended to poliomyelitis-infected hamster brain and spinal cord, and Ehrlich ascites tumor cells infected with West Nile encephalitis virus. These gave infective ribonucleic acid preparations (again of the order of 0.1 per cent of the control), but the ribonucleic acid from Bunyamwera-infected cells was not infective.[31] With infectivities of such a low order it is not sufficient merely to state that preparations are protein-free without at the same time giving the criteria and the size of the sample employed for analysis.

We suggest, too, that before it is concluded on the grounds of chemical and physical properties that ribonucleic acid is the infective agent, experiments should be conducted in which very *small* quantities of intact infective virus are added to very much *larger* quantities of ribonucleic acid extracted from normal *uninfected* tissues. It would be most surprising if the over-all chemical and physical properties of the mixture were not those of the major component—and, of course, if the infective properties were not those of the minor component!

These words of caution must now be applied to the claim of Hays, Simmons, and Beck[70] to have induced mouse leukemia with purified nucleic acid preparations. Cell-free extracts of AkR leukemic tissues were injected into newborn mice of two low-leukemia strains (C_3Hf/Gs and C57BR/cd) and into ($C_3Hf \times$

AkR)F_1 hybrids. The results confirmed the basic observations of Gross. It should be noted, however, that the incidence of spontaneous leukemia in the control hybrid mice was not low (7.7 per cent). In some AkR × C_3H crosses Law[78] observed a tumor incidence as high as 50 per cent! A mixture of deoxyribo- and ribonucleic acids prepared from nonleukemic and leukemic lymphoid tissue of AkR origin produced no leukemias in C_3Hf/Gs of C57BR/cd mice but 35.4 per cent in 31 of the hybrid mice. The mean age of development was, however, 2 months later than for the controls.

The evidence that we now have for the role of viruses in the carcinogenic process does not compel us, except in so far as we wish to be compelled, to believe that all tumors are virus-induced. However, recent virus research has shown that it is by no means inevitable, nor even usual, for the virus-infected cell to produce and liberate new virus. It has only been possible in recent years to begin to study the intracellular phase of virus infection, the phase in which the synthetic activities of the cell, or some of them, are directed toward the building of the specific patterns associated with the invader. Those viruses which multiply rapidly and are liberated in high yield are easier to study, and it is difficult to envisage a "future" for a virus whose replication becomes too cell-dependent. If, however, the cell itself is not destroyed but, instead, induced to proliferate as a consequence of the infection—and perhaps as a major consequence of the infection—then the virus may have secured its own "future." The difficulty about most of the tumor-producing viruses is that they appear to exist at the threshold of an extracellular state in cells in which they are produced at a very low rate.

The avian tumor viruses are, however, the fastest known carcinogens, and the importance for cancer research of detailed knowledge of their dramatic role in the conversion of normal to malignant cell cannot be overestimated.

I wish to express my thanks to my colleague Mr. P. J. Simons, to Mr. S. R. Scarfe, who prepared the sections illustrated in Figs. 6 to 9, and to Mr. K. G. Moreman, for photographing them.

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AN APPRAISAL OF TUMOR-VIRUS PROBLEMS

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INTRODUCTION

THE ability of viruses to cause cancer has been so amply documented since the pioneer work of Ellerman and of Rous as to be beyond debate. Spectacular advances in chemical and radiation carcinogenesis, that followed these discoveries, obscured research in viral tumors which, however, continued to make slow but steady progress. Viruses were discovered which were capable of producing tumors in plants, amphibians, birds, and several species of mammals. As knowledge has accumulated, tumor-virus problems have again gained prominence, and rightly so, for they touch upon the very problem of the nature of both tumors and viruses. Genetics has become linked to virology by the demonstration that self-replicating particles from bacterial cells are able to become incorporated into new cells, conferring upon these cells the genetic features of the original host.

Presently the concept of viruses varies with the discipline of the investigators and the kind of viruses they studied. Some microbiologists (as Burnet[26]) believe that viruses are microorganisms, some chemists (as Stanley[120]) that they are molecules. Both views are doubted by some investigators (Bawden and Pirie,[8] Lwoff[88]). Most geneticists tend to consider viruses as replicating agents of cellular origin. All of these views have merit and could conceivably be true. What are the facts and what do they mean from the standpoint of the origin of natural human and animal tumors?

Many excellent recent reviews and published conference proceedings adequately cover both past and current work on this subject.[3,12,28,38,70,82,95,126,127] This article is a personal appraisal of such major problems of current interest as: Are all tumors caused by viruses? What is the nature of viruses causing tumors? Are they infectious? In what way, if any, do they differ from infectious viruses? To what extent does animal experimentation with tumor viruses elucidate the broader problems of carcinogenesis? What is the meaning and significance of "masked" tumor viral infection?

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Attempts to answer such questions will follow a sketchy survey of historical events and some features of tumor viruses.

HISTORICAL SURVEY

Chronology of Some Major Findings, Related to Tumor Viruses

Chicken

1908—Leukosis agent (Ellerman and Bang[41])
1911—Sarcoma agent (Rous[102])
1933—Complex leukosis-sarcoma agents (Rothe Meyer and Englebreth-Holm[101])
1938—Ectodermal plaques on the chorioallantois of chick embryos (Keogh[75])
1940—Hemorrhagic disease by sarcoma agent (Duran-Reynals[36])
1942—Epidemiology of lymphomatosis (East Lansing Group[25])
1952—Characterization of erythroblastosis and myeloblastosis viruses (Beard and associates[12])

Human

1919—Human warts (Wile and Kingery[128])

Dogs

1932—Oral papillomatosis (de Monbreun and Goodpasture[34])

Rabbit

1932—Fibroma virus (Shope[115])
1933—Papilloma virus (Shope[116])
1935—Papilloma-carcinoma sequence (Rous and Beard[103])
1936—Fibroma-myxoma transformation (Berry and Dedrick[16])
1936—Oral papillomatosis of rabbits (Parsons and Kidd[96])

Mouse

1936—Mammary tumor agent (Bittner,[17,19] Andervont[3])
1951—Agent of spontaneous type of lymphoid leukemia (Gross[60,62])
1953—Parotid tumor virus (Gross,[61] Stewart[122])
1955—Lymphoid leukemia accelerating agent (Schwartz[109])
1956—Chloroleukemia agent (Graffi[56])
1957—Leukemia (reticulum cell type) virus (Friend[46])
1957—Broadening the range of parotid tumor virus by cultivation in vitro (Stewart[123,124])

Frog

1938—Kidney carcinoma (Lucké[85,86])
1952—Cartilaginous tumors in newts with Lucké agent (Rose and Rose[100])

Plants

1945—Wound virus tumors (Black[20,21])

Drosophila

1954—Tumor-inducing factors (Friedman and associates[68])

TUMOR VIRUSES

Evidence obtained on the morphology, biochemistry, and antigenic make-up of tumor viruses has not indicated any essential differences from the smaller classical (infectious) animal viruses. There have been no conclusive findings to indicate whether these viruses are free-living parasites or are laboratory-created from normal cellular particles.

Size.—The tumor viruses comprise a fairly uniform group ranging in size from about 30 to 70 mμ (Gross leukemia virus) to about 120 mμ (myeloblastosis virus). The following are reported estimates:

Gross leukemia virus	—	30-70 mμ[62]
Rabbit papilloma virus	—	44 mμ[83,114]
Rous sarcoma I virus	—	89 mμ[73]
Erythroblastosis virus	—	100 mμ[112]
Myeloblastosis virus	—	120 mμ[11]

All tumor viruses examined to date have been spherical. The Rous sarcoma virus,[42] the erythroblastosis and myeloblastosis viruses,[14] the rabbit papilloma virus,[83] and the Lucké kidney agent[44] have been shown to possess a complex internal structure similar in general nature to that of the vaccinia[27] and fowl pox[27] viruses.

There is a striking structural uniformity within the group of chicken viruses, suggesting that these viruses are interrelated.

Chemistry.—Difficulties in purification and concentration of tumor viruses have hindered progress. They are complex, having nucleic acid associated with protein as a minimum, and possibly other chemical compounds in unknown quantities.[12] If the material analyzed by Beard and associates,[10,11] the best thus far, is pure avian myeloblastosis virus, then it is far more complex than the plant viruses, possessing a high per cent (30 per cent) of fat, a low concentration of a nucleic acid (less than 9 per cent), at least one enzyme, ATP-ase, and three antigens (see below).

Based on analogy with non-neoplastic viruses one might expect tumor viruses to be comprised, in their simplest form, of DNA or RNA, and a protein. Viral replication and most of specificity might be expected to come from NA and serologic reactions from the protein, notably if the latter is coating the virus. Cross reaction with the host protein, if not a contamination, may mean incorporation of certain host proteins in the virus protein without complete breakdown and resynthesis, as has been reported for influenza virus.[1]

Immunology.—Earlier work by Andrewes[6] has clearly shown, by means of neutralization tests, a close relationship between various strains of chicken tumor viruses. Only the Fujinami duck virus appears to be markedly different from the other sarcoma viruses. The question whether sera against normal chicken tissues also possess virus neutralizing antibodies has been debated.[72,106]

In earlier experiments,[72] rabbit antisera against normal chicken spleens failed to neutralize a chicken tumor virus, while antitumor sera possessed strong neutralizing antibodies which were unrelated to complement-fixing antibodies.

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The presence of Forssman antigen and Wasserman hapten was also noted in the high-speed sediment from both normal and sarcoma tissues.

Beard and colleagues,[9-12] using purified virus concentrates, confirmed the findings on the antigenic relationship between the sarcoma and three types of leukosis viruses. Their purified virus of myeloblastosis possessed three antigenic components: one specific for the virus, a normal chicken antigen, and the Forssman antigen. Their erythroblastosis virus contained a specific viral antigen and a normal chicken antigen but lacked the Forssman antigen. Although the work of Beard and associates[11] indicates a high degree of purification, most of their conclusions are still open to some doubt. It is possible that the Forssman antigen in their virus concentrate is due to contamination with normal chicken material. Rubin[106] finds no compelling evidence that normal host components form an integral part of the Rous sarcoma virus structure. Rabbit "antichick" sera reduce virus titer by suppression of host cell multiplication. Such antibodies do not exist in the natural host. Since this virus is intimately related to the erythroleukosis virus, and one virus is known which is capable of producing both diseases, it seems unlikely that the "pure" erythroblastosis virus contains normal chicken proteins. The presence of neutralizing antibodies in animals that have been inoculated with sedimentable material from normal tissues could conceivably be due to the presence of virus in normal tissues. This is by no means far-fetched, in view of the prevalence of leukosis in chickens and the presence of neutralizing antibodies and infective virus in older, apparently normal, birds. Consequently, the high-speed sediment from pooled sera might contain sufficient quantities of virus to produce neutralizing antibodies.

Differences in ATP-ase activity between erythroblastosis and myeloblastosis viruses[9] can possibly be due to differences in concentration of the virus, since the myeloblastosis virus material was about 10 times more active than the erythroblastosis material. In centrifuged myeloblastosis leukemic blood, the plasma may contain much leukocyte debris from the thick buffy coat. This debris is more scanty in plasma from erythroblastosis blood, which is leukocyte-poor. In neutralization tests, erythroblastosis and myeloblastosis viruses were indistinguishable.[11] If the myeloblastosis virus material contained much chicken antigen, as demonstrated by immunization of rabbits, it remains to be determined whether the virus was contaminated with high-speed sedimentable nonviral chicken material, or whether the chicken material became incorporated in the virus framework during replication, either accidentally or by design.

Burnet[27] has commented on the absence of soluble antigens in tumor viruses and the relatively large component of host antigen as being indicative of a relatively recent *cytoplasmic* derivation of tumor viruses. However, recent observations on such classical viruses as the influenza virus[1] have revealed the extent and variability of incorporation of host protein into the virus particle even with an obviously parasitic virus.

A historical reconsideration of isolation of chicken tumor virus may be appropiate here. The high infectivity of virus in serum of chickens has long been noted.[48] Ultraspeed centrifugation of this serum yielded a pellet with high viral activity,[71] but, surprisingly, similar centrifugation of normal serum yielded a

pellet of about half that weight with no viral activity.[71] Technical advances have made possible further purification and concentration of the virus, but in the opinion of Bryan,[23] a tumor virus has never been obtained in greater than about 1 per cent purity. In light of this, a skepticism is justified in considering the electron microscopic pictures published as depicting viruses, with the possible exception of the purest leukosis virus preparations of Beard.[11]

Incidentally, the technique of fractional high-speed sedimentation originated by Claude[30] for this work, led later to isolation of inframicroscopic cellular units such as mitochondria and microsomes, and to identification of enzymatic activities attached to them. Phosphatases, Forssman antigen, and Wasserman hapten were the first so recognized.[53] The high-speed sedimentable material present in normal chicken plasma has not been identified. It is not inconceivable that this macromolecular material includes even DNA and RNA complexes. This is an illustration of how pursuance of an almost academic research on avian virus neoplasms led to discoveries reshaping the direction of cytochemical research. Claude's original concept that the tumor virus was but a modified normal cellular constituent,[30] then more intuitive than factual, may ultimately hold true for many tumor viruses.

Ecology.—Natural spread of some tumor viruses has been adequately documented. In the case of visceral lymphomatosis, evidence of natural infections has been presented by the East Lansing group of investigators. Natural infection with lymphomatosis virus may occur through the egg, or by exposure to virus shed from infected birds in the feces or saliva.[25] Since epidemics of visceral lymphomatosis occur in chickens throughout the world, the evidence is strong that this virus pre-existed in nature. This disease is said to have become much more prevalent since chickens have been mass produced in brooders. The other types of leukosis are highly "infectious" by artificial inoculation but do not appear to be contagious in nature. The Shope fibroma virus and the squirrel fibroma virus appear to be contagious in nature by direct contact and are perhaps also transmitted by insect vectors.[77,78] Mouse mammary tumor agent may also be naturally infectious since transmission from mother to offspring via the milk has been adequately demonstrated as a natural biologic process.[2-5] Although usually transmitted via the milk,[2] infection may occur from male to female during intercourse, even when the uterine horns of females are amputated and the males are vasectomized.[91] The male mouse harbors the mammary tumor virus in the testes, spermatozoa, vesicular glands and fluid, and in other sites.[4] Male secretions therefore, may be a source of infection to female offspring, either by direct contact or indirectly by infecting their mothers.

If the infective dose is low, a viral infection may remain latent. About 0.1 ml. of milk from a high mammary cancer strain is sufficient to infect susceptible babies up to 48 hours of age.

Bernhard suggests[13] on the basis of electron-microscopic studies that every strain of mice (but not rats or man) carries the mammary tumor agent—the agent being present in greater concentration in the high cancer strains. The existence of high and low cancer strains may be due to genetic differences in facilitating multiplication of the mammary tumor agent. A high infective dose can cause

tumors in resistant strains, the virus being passed on to successive generations for a time but the concentration of the virus eventually falls below that required for overt infection.

The situation is less clear with the mouse leukemia agent (Gross) and the parotid tumor virus (Gross-Stewart). It seems to us that the latter pre-existed before laboratory investigation and that vertical transmission of it occurs in C3H mice. With the Gross leukemia agent, this may also be the case, but it is equally tenable that it is "man-made."

The occurrence of *epidemics* or endemics of a neoplasm in many species is common knowledge. The explanations vary. Genetics, common exposure to an extrinsic carcinogen, or viruses have been blamed. All are conceivable. Notable in this respect are the venereal sarcoma in dogs and the leukemias in cattle and fowl. Only in the latter do well-designed epidemiologic experiments prove natural transmission.

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Venereal sarcoma[74] is a remarkable disease of dogs that spreads by intercourse and thus can cause epidemics in kennels. One apparently metastasizing tumor regressed following subtotal resection while in several genetically unrelated dogs tumor grafts caused fatal disease with disseminated tumors.[125] The possibility that this is a viral tumor deserves renewed research.

The role of *antibody* formation in response to natural or artificial infection with the tumor viruses and its relation to spread of the viruses and tumor formation is poorly documented and not well understood. Regression of Rous sarcoma can occur in association with a rising antibody titer. Chicken tumor viruses and circulating antibody can coexist in the same chicken and the presence of neutralizing antibody does not seem to influence development of virus-induced tumors, their regression or the subsequent infectivity of the chicken.[38]

Classical viruses produce, in general, a short-lived disease with lasting immunity. Most virus tumors progress slowly, immunity phenomena appear entirely absent in some (as mammary tumor agent, Gross leukemia virus) or are merely suggestive with others. This might be explained by postulating that tumor viruses enjoy an intimate relationship with the host cells and so may escape destruction by antibodies. True, they can be free in the plasma in high concentration, but free virus may be unimportant as concerns their perpetuation in the infected hosts. On the other hand, it can be postulated that tumor viruses are poor antigens because of the chemical closeness of virus and host proteins. The available data leave this question open.

With many tumor viruses the *age* of the host is of critical importance—e.g., mammary tumor agent and Gross leukemia agent. Usually, as with classical viruses, the younger the animal the more susceptible it is to infection, although not necessarily to tumor formation. However, the erythroblastosis virus is said to be more pathogenic for older chickens than young.[11] The basis for this striking dependence on host age is unknown, but it may have certain implications for the nature of these viruses.

If one accepts that tumor viruses are true parasitic organisms, then the strikingly long *latent period* between infection and tumor formation by some of these viruses needs consideration. The work of Burmester and colleagues clearly

demonstrates that the chicken leukosis viruses can infect their hosts and pass from one host to another without tumor formation.[25] When the infective dose is relatively small or antigenicity is poor, an infection is likely to remain inapparant or permanently latent, with no resulting tumors. Since infection can occur without subsequent tumor development, it is possible that tumor formation in response to infection is a merely incidental event in the usual host-parasite interrelationships of tumor viruses.

With tumor viruses, there is meager information available on changes in *virulence*, characteristic features of classical viruses. Apparent changes in virulence in Rous sarcoma virus are attributed by Bryan and associates[22] to changes in concentration of the virus.

Recently Gross[63] reported markedly enhancing the infectivity of his mouse leukemia agent. By successive passages in susceptible hosts this agent acquired ability to produce leukemia in older than day-old mice, in a larger per cent of injected animals, and after a much shorter latency period than the original agent. Aside from its practical value, this phenomenon, if confirmed, would be of unusual theoretical interest.

SPECIFICITY, VARIABILITY, ADAPTABILITY

The following properties appear to distinguish the tumor viruses from infectious viruses: species, strain and cell specificity, and dependence on age of the host for pathogenicity. Whereas "classical" viruses can infect widely distant species, tumor viruses can infect only closely related species. There are exceptions: measles, varicella, and rubella are limited in their host range. Limited cytotropism is a characteristic feature of all viruses but appears to be more narrow in tumor viruses. Some viruses (as that of the Shope papilloma) will not even affect the normal resting cell and tumor formation is greatly enhanced by nonspecific proliferative stimulation of the virus receptive cells. (Rous and associates cited in Oberling and Guérin.[95])

Broadening of cytotropism in embryo or in tissue culture is also a common feature to all viruses. Young animals are more susceptible than adults and often only newborn animals are receptive to tumor virus, with the reported exception of erythroblastosis.[11]

It is difficult to evaluate many reports violating these tenets. The material used was often not cell-free and cells notably of embryonal type, can be heterografted.

Modifications occur when virus encounters an abnormal (unfavorable) environment. Proliferation of virus appears least inhibited in embryos and in very young animals. The greater the deviation from the original environment, still permissible with survival, the greater the modification of the virus.

Passage through young ducks not only adapted the Rous chicken virus to ducks but made it pathogenic for the pigeon.[38] Turkey-adapted Rous virus lost its infectivity for chicks.[37] Rous virus, passed through ducks, lost virulence for chickens and acquired it for ducks. When passed through the anterior chamber of the eye, it acquired ability to produce periosteal tumors.[117] The leukoses viruses affect only gallinaceous birds.

The frog virus of Lucké can acquire skeletal and iris affinity after passage in tadpoles and salamanders. This acquired affinity of Lucké's virus is thought by Rose and Rose[99,100] to be comparable to genetic crossing-over.

The host cell range for tumor induction of viruses of papilloma of rabbits, mammary tumor, and leukemias of mice is limited to species and cell type of origin. Of special current interest are the viruses of mouse leukemia, notably because of similarities and reported links to human leukemia. The virus of Gross is thought to be the cause of spontaneous leukemia in the high leukemia strain AK. The occurrence of sarcomas, parotid gland,[61,122] and adrenal tumors[122] has also been described in mice injected with this virus, but it seems to us that the agent causing these tumors is different from that which produces leukemia.

The parotid tumor virus seems to be indigenous in C3H mice, in which it occasionally causes tumors. Its virulence (or concentration) is enhanced moderately by animal passages, as done by Gross[63] and Dulaney[35] and greatly by the use of tissue cultures, as done by Stewart and associates.[123,124] This reasoning is based on the following: (a) Parotid tumors were not seen in our AK colony, not even when large numbers of newborn mice were inoculated with tissue extracts from Ak mice.[52] C3H mice are susceptible to both leukemia and parotid tumor viruses. When Ak leukemia virus passaged in C3H mice was used as a source of virus, Ak mice proved receptive to parotid tumor virus.[24] (b) The parotid tumor virus is of smaller size, and when the mixture of the two viruses is filtered through membranes with pore size 93 to 140 mμ, the filter contains only the parotid tumor virus; no leukemias were induced in newborn C3H mice, which are highly sensitive to the leukemia virus.[62] It is unlikely that one virus could be split into two so different infectious units. (c) The leukemia virus is destroyed by ether, the parotid tumor virus is not.[62] (d) The virus recovered in tissue cultures has predominantly parotid gland affinity and produces no leukemias.[123]

The parotid tumor agents grown in mouse embryo tissue cultures produced as many as twenty-three different, presumably primary, neoplastic lesions in mice inoculated shortly after birth with supernatant fluid of tissue cultures.[123] All mice that developed neoplasms had parotid gland tumors. In addition, many had adenomatoid lesions in the kidney, and one or more tumors in other organs, e.g., salivary glands, lacrimal gland, thymic epithelium, adrenal medulla.[123] Mixed tumors of the parotid gland appear in a wide range of histologic types and it is often difficult to distinguish with certainty primary from secondary tumors. The wide range of affinity of this virus is without parallel among the well-studied tumor viruses.*

This virus is also unique in crossing the species barrier, producing sarcomas in hamsters.[40] These observations are not yet conclusive, since thus far only unfiltered virus-infected fluids from tissue cultures of mouse embryo cells were used. Although similar noninfected embryo cultures did not produce tumors, experiments of Greene[57,58] have shown that cells infected with rabbit tumor virus are heterotransplantable while similar normal cells are not. Furthermore, Foley

*The distinctness of leukemia and parotid tumor viruses is also suggested by current experiments of Buffett and associates in which supernatant of Ak leukemia extract centrifuged at 105,000 × g for 35 minutes caused parotid and other tumors in 23 of 30 injected newborn Ak mice within 8 months, but no leukemias. The tumors were of the type produced by Stewart and her colleagues in mice injected with tissue culture material infected with similar virus. Thus, tissue culture is not essential to disclose the multipotentiality of this agent.

and Handler[45] succeeded in producing tumors in the cheek pouch of adult hamsters by injection of large numbers of several types of normal human cells grown in vitro. The point at issue is whether the tumors produced by Eddy and colleagues in the newborn hamsters were composed of hamster cells or mouse cells. These tumors were not transplantable to adult hamsters.[40]

Several studies indicate that *proliferation of the tumor agent* is supported by many cells of susceptible hosts, but neoplastic stimulation commonly occurs only in one cell type. Pikovski[98] demonstrated an increase in titer of mammary tumor agent in tissue cultures of chicken fibroblasts. In vivo, this virus produces tumors only in the mammary gland. Rous sarcoma virus causes carcinoma-like nodules on the chorioallantois, but no carcinomas in adult hosts.[75] The neoplastic nature of these lesions is debatable.

INTRACELLULAR INFECTIOUS PROCESS

Following infection of a cell with a classical virus such as influenza, an orderly, well-defined sequence of events occurs.[27] There is an initial eclipse phase during which the virus breaks down into numerous component parts. This is followed by a period during which replication of the components is carried out by the host cell under the direction of the viral fragments. Then follows a period of reassembling of the synthesized progeny virus with subsequent release of the progeny from the cell either as a "burst" (vaccinia) or a reasonably simultaneous extrusion through the host cell membrane (influenza).

In association with these events, the host cell may show changes ranging from cell necrosis (polio) to giant cell formation (measles) or, as in the case of the pox viruses, a limited and fairly regular cell proliferation with no invasive or tumorlike features.

It is likely that in the intracellular events following tumor virus entry lie many of the clues to the mechanism of virus-induced carcinogenesis. Progress has been hampered by the difficulty in obtaining highly susceptible host systems suitable for quantitive work of this type. The nearest approach to a satisfactory system has been the use of the chorioallantoic membrane for studies with the Rous sarcoma virus by Rubin[105] and the use of the chicken embryo brain—Rous sarcoma virus system by Groupé and Rauscher.[65,67,84]

A variety of end results can follow infection of host cells by the same tumor virus. In the case of the avian tumor virus carriers or mammary tumor agent in young female mice, it is possible to have virus multiplication in the absence of histologic lesions. With the Rous virus, depending on the age of the host, infection with virus multiplication may occur either in association with a hemorrhagic disease,[36,67] or with sarcoma or fibroma formation.[38]

The "hemorrhagic" and "hepatic disease" is attributed by Groupé and associates to an increased concentration of the virus.[67] However, the microscopic changes suggest that this is not a fundamentally different disease, sarcoma-like cells being abundant in the lesions.

It is possible that differences in the mechanism or rate of intracellular replication of tumor viruses may occur even with the same virus-host cell system depending on the route or size of infecting dose and the metabolic state of the host cell. This may cause considerable variation in the subsequent behavior of the

host cells. Alternatively, the processes of virus replication may be identical in all systems, and may not be directly carcinogenic, but be indirectly connected with carcinogenesis by secondary mechanisms existing in certain host cells under certain metabolic conditions.

Fragmentary observations suggest that the processes of tumor virus replication may differ from those of other viruses. Harris[69] was unable to demonstrate an eclipse phase following entry of Rous sarcoma virus into chorioallantoic membrane cells. Similarly, on the basis of electron microscopic evidence, Claude and associates[31] suggested that the Rous sarcoma virus appeared to divide by binary fission rather than by the replication process usual for classical viruses. Such evidence, if confirmed, would imply that tumor virus replication was more akin to replication of cytoplasmic particles (i.e., binary fission) than to that of classical viruses for which the eclipse type of multiplication is now considered characteristic.

However, the electron microscopic pictures of Fawcett[44] of cells containing the Lucké agent and those of Epstein[43] of Rous sarcoma cells show complex nuclear and cytoplasmic structures similar to those seen in cells with replicating nontumor viruses. Fawcett has also observed a complex cycle of replication and reassembling of the Lucké agent which strikingly resembles the proposed system for the influenza and vaccinia viruses.[27] Similar observations have been made by Bernhard and Oberling for the Shope fibroma virus.[15] They trace the development of this virus to a finely granular cytoplasmic mass, named viroplasm. This material is antigenic, but at the six-hour stage after infection contains no sedimentable virus.

It appears[105] that under some circumstances Rous sarcoma cells may slowly release replicated virus over prolonged periods. This differs from the sequence usual for classical viruses and may indicate either a protracted replication cycle or the possibility of multiple replication cycles within the same host cell.

There appears to be a marked difference to superinfection with other viruses between the behavior of cells infected with classical viruses on the one hand and tumor viruses on the other hand. Whereas cells infected with classical viruses do not usually support the multiplication of a second unrelated virus, virus-induced tumor cells can support multiplication of other viruses. Newcastle disease virus and vaccinia virus can multiply in Rous sarcoma cells.[118] Indeed, in some instances, infection with tumor viruses appears to confer susceptibility to virus infection on otherwise resistant cells. Semliki Forest virus fails to proliferate in normal rabbit fibroblasts, while it can grow in fibroma cells of the virus-induced Shope fibroma.[55]

Thus the question of whether tumor virus replication differs significantly from replication of other viruses still remains unanswered.

It also remains to be proved that host cells multiply following *any* virus infection. The mere observation that cell proliferation occurs following virus infection is not sufficient. Conclusive proof would be (1) that a single cell which is known to contain virus can multiply and later produce virus, or (2) the demonstration of an inclusion body in a cell with a mitotic figure. This problem is more pertinent to tumor viruses where not only cell proliferation but also cell neoplasia is involved.

Since it is already known that tumor virus multiplication can occur without obvious cell proliferation, it is important to demonstrate the direct association between tumor virus multiplication and neoplastic proliferation of the same cell. If this cannot be demonstrated, then viral carcinogenesis may be no more than conditioned carcinogenesis in neighboring cells due to products from non-neoplastic virus-infected cells, or the presence of a neoplastic virus within a tumor cell may be the result of a parasitization process *following* and not preceding neoplastic transformation. Observations of Epstein[43] have indicated that only one Rous sarcoma cell in 50 to 3,000 contains electron microscopic particles suggestive of Rous sarcoma virus. This was confirmed by concurrent biologic assays. Using the chorioallantoic membrane Rubin[105] was at best able to show that only one Rous sarcoma cell in five contained infectious Rous virus.

While it is unreasonable to expect that all virus-induced tumor cells should contain infectious virus at any one time, the suspicion remains that some virus-induced tumor cells may never have been infected and that replication of tumor viruses may be only indirectly related to induction of tumors or to maintenance of tumor cells.

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TUMORS AND TUMOR VIRUSES

Mechanism of Tumor Formation.—The long *latent period* between virus infection and tumor formation has been interpreted by some investigators as indicating that the tumor viruses can persist for long periods in an eclipse phase or a "pro-virus" state. The evidence from the chicken viruses and the mammary tumor agent, however, indicates that replication of these tumor viruses does occur during this period. Delay in tumor formation must therefore be ascribed to environmental factors in the host.

Under optimal conditions, as with the Rous virus on the chorioallantoic membrane[105] or the Friend virus in young adult animals, [90] tumor formation occurs within a few days following infection. This suggests that neoplastic proliferation in the host cell can occur almost simultaneously with initial virus replication, but in most cases neoplastic proliferation in the host cell is apparently delayed until accessory factors render a host cell especially responsive to the virus or stimulate virus-infected cells (Rous and Kidd[76]). There is also the technical difficulty in identifying neoplastic cells before they form definitely identifiable tumors.

Susceptibility is enhanced by proliferative stimuli regardless of type. Such stimuli are apparently maximal in the embryo. Enhancing agents recorded are: tar, chemical carcinogens, Scharlach R, simple trauma.

Causation of a neoplasm by a virus can be conceived by several different mechanisms, and there is no more reason to suppose that only one mechanism exists than there is to assume that all viruses are alike. In the following, three theories will be discussed: conditioning, mutation, and transduction.

Conditioned neoplasia:* Viruses could drive parasitized cells to proliferation without destroying them and without causing a neoplastic mutation in the parasitized cells. The following observations suggest this possibility: (a) failure to isolate virus-free tumor cells from such viral tumors as chicken sarcoma and

*In Greene's terminology,[59] dependent (or conditioned) tumors, in contrast to autonomous tumors, are those that are not heterotransplantable. In our terminology, the distinction is based on behavior in normal isologous hosts. Growth of dependent tumors in isologous hosts is conditioned on some correctable disturbance in growth-regulating or influencing forces of a cell. Autonomy is conceived as due to an irreversible chromosomal change.

leukosis, (b) populations of tumor cells caused by several viruses lack the anaplastic features of autonomous cancers, e.g., it is not possible to distinguish a cell population of erythroblasts in erythroleukosis from that obtained by a hemolytic agent as phenylhydrazine.[47] In tissue culture, normal fibroblasts cannot be distinguished from sarcoma cells in the light microscope[48] but in chickens one causes a tumor, the other does not. Rous and others hold that the sarcoma viruses are not only initiating, but also maintaining causes of the tumor. Such viral tumors would be conditioned neoplasms. Theoretically, disappearance of the virus from tumor cells (spontaneously or induced) could allow the host cell to revert to its normal prototype.

The mechanics of conditioning by viruses can be explained as follows: it is possible that the tumor virus interferes with production of a growth-regulating substance. By a process analogous to a *deletion*, such cells might then multiply unrestrained. Alternatively it can be supposed that the virus blocks the response to extrinsic physiologic cell-restraining factors.

Mutagenesis: Viruses (conceived as submicroscopic parasites) could cause a somatic mutation in cells by different mechanisms, and either persist in or vanish from cells. In this sense viruses would behave as radiation or chemical mutagens. The example of rabbit papilloma-carcinoma transformation can be cited,[103] the virus being abundant in the former, persisting for some time in the latter, ultimately disappearing, as indicated by all criteria including lack of viral antibodies in the carcinoma-bearing rabbits.

Transduction: It is conceivable that some tumor viruses are genic complexes derived from either neoplastic or potentially neoplastic cells. This theory seems to apply to the Gross virus. In favor of this[52] are the long latent period, the limitation of infection to genetically compatible strains and to the very young animal or embryos; also, the fact that the disease produced closely resembles in character and time of onset a spontaneous disease which is proved to be determined by genetic factors. Confirmatory evidence as chemical features corresponding to NA, response to enzymes, e.g., inactivation of the transmitting agent by NA-ase, has not yet been reported. This theory would also imply that thymectomy should prevent "viral" induction (transduction) of this leukemia but not viral multiplication and genetic transfer of "virus" from parent to offspring.[52] If the thymus is merely the residence of the leukemic virus, thymectomies in successive generations of hosts may ultimately reduce the incidence of "spontaneous" thymic lymphomas in the high leukemia strain AK. In contrast, if the incidence of spontaneous leukemia is truly genetic and thus dependent on continuity of germ plasm, this procedure would not reduce leukemia incidence in the offspring unless genetic continuity is influenced by somatic "organizers."

The transduction theory has several variants as (1) Acceptance of iso-NA, (2) Acceptance of hetero-NA. This would be carcinogenesis by *addition*. According to this hypothesis, what is added is an unrelated genetic component of extrinsic origin, i.e., exchange or addition of "viral" nucleic acid, derived from another cell of the same kind, already possessing a genetic liability to neoplastic transformation. This process would be in effect similar to well-known transformation or transduction in bacteria. A similar idea has been advanced earlier by Murphy[92] for chicken tumors, but he related this process to mutation and

named the agents "mutagens." (3) Acceptance of extra chromosomal determinants, i.e., replicating units other than nuclear genes but containing NP. Similar nonchromosomal transmission of features of cells can be brought about by bacteriophage, kappa particles of paramecium, genoides of Drosophila, and transforming agents (NA) of bacteria.

The possibility that injected NA or NP may be built into the nucleus by a mechanism of physiologic exchange has been suggested by experiments with liver chromatin.[89] This may lead to change in function at the locus of chromatin exchange comparable to gene mutation. Experiments with liver tumor and lymphosarcoma suggesting such a possibility were reported by Stasney and associates,[121] but the possibility of contamination of the chromatin with living cells has not been excluded.

Replicating filterable material acting as a transducing agent might conceivably behave in nature as an infectious "virus," but foisting bits of alien NP or NA into replicating cells is more likely to be an artificial laboratory maneuver than a natural process.

Theory 1 (conditioned neoplasia) might yield a cell with viral inclusion, more likely in the cytoplasm than in the nucleus. Nuclear anaplasia is unlikely. Theory 2 (mutation) might yield a cell without viral inclusion but with nuclear anaplasia. Theory 3 (transduction) might yield a cell without abnormal cytoplasmic bodies and with or without nuclear abnormalities.

Application of the state of affairs with bacteriophage would perhaps resolve all basic tumor-virus problems, but does such an analogy hold? Bacteriophage appears to be capable of transforming into cell constituents and vice versa; it can mutate, giving rise to viruses which carry genetic factors in their host cells.[87] Experiments based on the analogy of bacteriophage have not been fruitful, e.g., radiation does not result in "unmasking" of leukemia virus from leukemia cells.[79]

Virus as co-carcinogen: Factors which influence the state of the cells can be the deciding force in determining whether or not a tumor will eventuate following viral infection, e.g., estrogenic stimulation conditions induction of breast tumors by mammary tumor agent.[3] Conversely, a viral infection can promote tumor formation in "initiated" cells. The following is a likely example. Intradermal injection of *vaccine* virus into skin of mice prepared by painting with methylcholanthrene, combined with injections of cortisone, is frequently followed by the development of neoplastic lesions at the sites of injection.[39] Similar lesions have never developed in animals that received this carcinogen or the virus alone. The likely explanation of this phenomenon is that methylcholanthrene initiated the lesion, and vaccinia virus acted as a *promoter*. Methylcholanthrene, as radiations and probably other mutagens, can cause silent alterations which become manifest when a second proliferative stimulus such as a virus is applied. The co-carcinogenic effect of virus has been well documented by Rous and associates in numerous well-designed experiments.[95]

Features of Avian Mesenchymal Tumors.—Avian tumor neoplasm may furnish the key to may tumor virus problems. They are remarkable in several ways: (1) when searched for persistently by laboratory experimentation, practically all mesenchymal tumors yielded a virus; (2) natural contagion has been established

with one, the most common type, but only after intense large-scale laboratory experimentation; and (3) the natural history and anatomic features of the avian neoplasms in no way differ from those of mammalian neoplasms.

The avian leukoses are best classified by the type of cell affected. "Avian leukosis complex" is a popular but meaningless term. Erythroblastosis and myeloblastosis are rare natural diseases but are readily transmissible and yield the virus. Lymphomatosis occurs in several forms and is probably caused by several different viruses.

The discoverer of fowl leukosis (Ellerman) believed that all types of leukosis of the fowl are caused by a single virus. Subsequently, viruses were described which caused only erythroleukosis, others more commonly, erythroleukosis and myeloid leukemia. Later, viruses were described with ability to produce both sarcoma and erythroleukosis. Accordingly, they were classified as simple and complex viruses. Numerous earlier attempts to change a sarcoma virus into a leukosis virus and vice versa have failed. Nor has it been possible to isolate several viruses with distinct properties from strains which were capable of infecting different types of cells. This led to the concept that there are many avian tumor viruses; some stimulate a single type of cell, others related types of cells. Among the complex viruses, one produces predominantly erythroleukosis and lymphomatosis, another erythroleukosis and sarcoma. Recent literature has not clarified this situation. At one extreme are the observations of Carr,[29] showing the ability of a complex virus (virus ES4, which produces erythroleukosis and sarcoma) also to cause adenocarcinoma of the kidney when injected into young fowls. The latter is a unique observation. Earlier studies[93,94] suggested this possibility, but the experiments were inconclusive. Spontaneous epithelial neoplasms are not uncommon in this species. Among thousands of young fowls infected with avian leukosis virus by many investigators, no adenocarcinoma was produced. At the other extreme are the findings of Beard and colleagues[12] who assume that erythroblastosis and myeloblastosis are caused by different viruses. If so, the question arises, why do these two usually go together? Frequency of their association suggests that they are variants of the same virus.

It is puzzling why the erythroblasts in erythroblastosis are normal appearing, lacking all features of a cancer cell. This suggests a conditioned growth disturbance. Unlike leukemia cells in human myeloid leukemia which occur in a variety of types, the myeloblasts in myeloblastosis of chickens are monotonously alike, the predominant cell resembling a nonmalignant monocyte. This, too, favors the idea that this is a virus-conditioned growth disturbance.

A rare but characteristic type of leukosis of chickens is myelocytomatosis in which myelocytes form disseminated tumors in bones. This has never been reproduced in the laboratory.

The most common type of spontaneous leukosis is the aleukemic type of lymphoma, yet the first that yielded a virus[48] was of the rarer leukemic type.[49] Distinct from these is another common type of lymphoma with marked neural affinity (neurolymphomatosis) which is probably caused by a different agent.[11] A sclerotic change of the bone marrow, possibly a late sequel of some leukoses, is osteopetrosis. The pathogenesis of this disease and its relation, if any, to leukosis has never been thoroughly elucidated.

The complex virus strains recorded cause a combination disease of some type of leukosis with some type(s) of sarcoma. The individuality of these viruses is clearly indicated by their cellular affinities. Even when affecting one cell type the character of tumor is determined by that of the virus. For example, a virus described by Rous, Murphy, and Tytler[104] caused fibroblasts to form bone. Another virus[38] had to be brought in contact with osteoblasts to produce osteogenic tumors, and it failed to stimulate common fibroblasts. Similarly, the special histologic features of fibroblastic tumors are virus directed.

Decades ago many experiments were performed to adapt viruses to different types of cells, with contradictory results. Rous virus after passage through the following alien hosts produced periosteal sarcomas: (a) turkeys,[37] (b) guinea-pig eye,[117] (c) conservation in vitro or in vitro culture.[38] This is a minor modification. The range of this family of viruses includes leukoses and sarcomas including osteosarcoma and endothelioma. The possibility that the virus was complex at the start has not been excluded. Modern techniques such as growth in tissue culture may disclose greater adaptability of this family of viruses than suggested by older work.

Unique Features of Mammary Tumor Agent.—The mouse mammary tumor agent has shown the following remarkable features: (a) it is transmitted via the milk and can infect nonhistocompatible strains of mice; (b) ease and persistence of subsequent virus multiplication is determined by the host's genetic make-up; (c) transmission of the disease is neonatal or even congenital but not genetic; (d) resistance is rapidly acquired with age—restraining tumor formation but not preventing viral multiplication, and this is not explicable on an immunologic basis; (e) viral tumor induction is markedly dependent on hormones, and mammary tumors are rare in virus-infected males; (f) the virus is probably able to multiply in cells other than mammary epithelium but incites tumor formation only in the latter; (g) the virus can induce tumors in most or all strains of mice but not in other species; and (h) characteristic subcellular particles are present in the mammary glands of mice of all strains studied, but absent in the mammary gland of rat and man.[13]

Some Features of Mouse Leukemia Viruses.—To Gross belongs credit for demonstrating, following failure of many other investigators, that murine neoplasms can be induced by a viruslike agent. Subsequently, several viruses causing leukemia and tumors in mice have been described, and it now seems that the story of avian neoplasms will be repeated with murine neoplasms, thus markedly closing the gap to recognition of similar tumors in man.

A healthy skepticism is voiced by Law[81] of categorical statements that "spontaneous mouse leukemia is caused by an infectious virus," that it is an "egg-born virus disease," and that an extrinsic leukemia virus by "vertical transmission" has been established on genetically unchanged soil (the C3H mouse). We consider the evidence strong that cell-free replicating agents can induce leukemia in mice and that, in the case of the Gross agent, the induced disease is indistinguishable from the spontaneous disease. Ability to hasten leukemia development alone, as Law remarks, is not proof of a virus but the reports of Schwartz and Gross suggest replication of the agents concerned. More evidence of transmission of virus from injected mice to their offspring is desirable.

Gross[62] noted that cell-free material from leukemias of high leukemia strains caused three types of neoplasm in C3H mice: lymphoid leukemia, parotid tumors, and fibrosarcomas. (Adrenal tumors reported by Stewart were not noted by Gross or by us). Although he obtained some increase in incidence of one type of neoplasm by using homologous tumors as the source of virus, a separation into three distinct virus strains was not accomplished. However, the technique used may not allow separation of three distinct but related viruses. There are indications that parotid tumor virus is a distinct entity, and these are stated previously. The leukemia virus appears to act genetically in the host cell and may be a transducing principle.[52] The parotid tumor virus is characterized by low infectivity, rapid "masking" of this virus in tumor-bearing hosts, extraordinary unmasking, and increase in virulence (or concentration) in tissue cultures.[123,124] The work of Buffett[24] has indicated the possibility that the parotid tumor virus may spread by cross infection from injected newborn mice to uninjected litter mates. This type of risk must be borne in mind in tumor virus transmission experiments using highly susceptible hosts.

Schwartz and associates[108] described two leukemia viruses. Filtrate of brain from leukemic mice of their Swiss strain listed in Table I produced leukemia in about 50 per cent of Swiss mice within about 1 to 3 weeks, while leukemic tumor filtrates from the same mice failed to induce the disease. Cell-containing material was no more effective than filtrate. The outstanding change was a massive mesenteric lymphosarcoma with hepatosplenomegaly, irrespective of the route of injection (i.c. or i.p.). This filtrate failed to accelerate leukemia in Ak mice. The disease illustrated[108] is unquestionably lymphoblastic leukemia. The extraordinarily short latent period, the lack of recovery of the virus from the lymphoid tumor and its recovery from the brain, are without parallel in the virus field.

TABLE I. COMPARISON OF FOUR DIFFERENT TYPES OF REPORTED LEUKEMIA VIRUSES IN MICE

INVESTIGATOR	STRAIN OF ORIGIN	ADULT MICE	CELL TYPE	STRAIN SENSITIVITY	ORGAN PRINCIPALLY INVOLVED
Gross[62]	Ak, C58	resistant	lymphoid (thymic)	C3H, Ak sensitive	thymus (?)
Friend[46]	Swiss*	highly sensitive	reticulum cell with erythroblastosis	C3H, Ak resistant	spleen
Graffi[56]	several‡	somewhat sensitive	myeloid	Bluhm, sg, db sensitive	bone marrow (?)
Schwartz†[108]	Swiss	highly sensitive	lymphoid (sarcoma)	Swiss sensitive Ak resistant	mesenteric node (?)

*Carrying transplanted Ehrlich carcinoma.

†This is the second agent of Schwartz. His first agent, found in the brain of leukemic man and mice, merely hastens leukemia development. Brain contains infective agent; leukemic tumors do not.

‡Graffi obtained infectious filtrates from diverse tumors (carcinomas and sarcomas) which produce myeloid leukemia but not the original disease.

The possibility that brain tissue might have rendered the filters permeable to cells has been excluded by the use of *Escherichia coli* for test of filters. According to earlier work of Schwartz and associates,[109,110] the brain of leukemics (man and mouse) contains a factor which accelerates the development of leukemia in Ak mice. This factor is absent in normal human brain, and Schwartz postulates that it is a virus. This far-reaching conclusion calls for stronger evidence than presented thus far.

The leukemia induced by the Friend virus[46] originates in a cell which is conspicuous in the pulp of the spleen and has the morphologic features of a reticulum cell. It is associated with marked nonmalignant erythroblastosis, without causing leukemic involvement of lymphoid or myeloid systems, differing in these and other respects from other leukemia viruses described (Table I). Observations suggest that the leukemic reticulum cell does not proliferate in the new host but yields a virus which infects other cells.[90]

Originally the virus of Graffi, as that of Friend, was isolated from Swiss mice bearing Ehrlich carcinoma. Graffi's virus is said to cause myeloid (chloro-) leukemia[56] which the other viruses do not. The origin and nature of both virus strains is uncertain.

Law[81] questions the neoplastic nature of the disease described by Graffi and colleagues,[56] Schmidt,[107] and Friend[46] because they are not transplantable in the strain of origin. This is a matter of limiting the term of neoplasm to those that are autonomous. In our view, Friend virus induces a virus-dependent neoplasm. Hormone-dependent neoplasms grow progressively and metastasize only in conditioned hosts.[50] The existence of such tumors necessitates re-definition of neoplasia as a state wherein certain cells proliferate progressively producing neoplasms due to changes either in the host or in the cell. We now recognize that neoplastic proliferation of cells can be due either to an irreversible change as a somatic mutation, or to a virus, resident in cells; the latter is probably reversible.

ARE ALL TUMORS CAUSED BY VIRUSES?

Unless viruses are identified with genic complexes of cells, to consider viruses as the common if not the sole cause of cancer is in glaring opposition to facts.

(1) Local x-irradiation of several organs results in tumors of the irradiated organs—such as pituitary, ovary, bones.[54] These tumors are usually transplantable in histocompatible strains only. Several cell types of the same ovary can give rise to tumors; upon transplantation each of these reproduces the same cell type.[51] Thus, in terms of the virus theory, we are bound to suppose that each of these cells has its own resident tumor virus. Furthermore, this implies that these viruses are not only strain- but also organ-specific and are masked so completely that their presence is undetected by any known procedure other than cell transplantation.

(2) The same argument applies to transformation of sterile embryonal cells into neoplastic cells by contact exposure to a chemical carcinogen.[119] Thus, to explain the origin of these tumors on basis of a virus resident in cells is without foundation; to identify their causative agents with genes is almost meaningless.

However, it is conceivable that fragments of neoplastic cells created by chemical carcinogens induce similar neoplastic transformation in normal host cells by a process of transduction, as described in lower organisms. But the speculation that such particles acquire an independent existence and behave as infectious units, although attractive, is unfounded. When malignant tumors develop following exposure to a chemical carcinogen or radiation, their maintaining cause is best conceived as a change in parts of the cells involved in their reproduction.

(3) Irradiation can induce tumors in most animals in a variety of organs; in female rats, total body irradiation with doses greater than 25 r, causes mammary tumors, the incidence rising with the dose to over 75 per cent.[33] This could mean that radiation activates a latent virus specific for the breast. Tumor induction is prevented by ovariectomy.[32] Presumably, transplantation of mammary tumors of rats succeeds only in histocompatible strains of the same species and only with cells. This situation is worthy of further careful analysis.

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(4) Tumors can arise at sites distant from the organ injured but in cells which are functionally related to the injured cells: e.g., destruction of the thyroid leads to thyrotropic pituitary tumors, hyperestrogenization to mammotropic and other tumors. Development of these tumors is often preventable by subsequent restoration of the deranged hormonal equilibrium. This type of tumorigenesis does not fit either the viral concept or that of somatic mutation but is readily explainable on the basis of a disturbance in normal homeostasis of the host. Many mammalian tumors appear to originate this way, with subsequent acquisition of some autonomy by the cells involved. It is probable, that a resident virus nonfatal to the cell could greatly enhance the likelihood of tumor development in such situations.

In search of such viruses, enhancement of tumor development by cell-free material should be sought for. The respective viruses need not exhibit genic identity with host cells. This seems to be the situation with the mammary tumor virus of mice which can affect cells of many strains which are histoincompatible with each other. The cells rendered neoplastic are histocompatible with the host of origin.

"MASKING" PHENOMENON AND IDENTIFICATION OF TUMOR VIRUSES

"Masking" of Viruses.—Designation of a tumor as viral can be made with certainty, but not that it is nonviral. In a partially resistant host (as chicken sarcoma virus in turkeys or the virus of cottontail rabbit fibroma in domestic rabbits) the tumor may appear to be nonviral unless a sensitive host is known. The parotid gland tumor in mice is transplantable with difficulty and does not yield a virus on direct tests. It is old experience that tumors presumably caused by viruses, but not readily transplantable, are not likely to yield a virus. It seems that the parotid tumors, as the rabbit papillomas, are not autonomous neoplasms but tumors conditioned by specific cell-bound viruses. When cultured in vitro with embryonal cells, they yield virus in abundance.[123] This tumor resembles closely human salivary gland tumors, and it is possible that the human tumor is also virus-induced.

Similarly, the mammary tumor virus of mice is inapparent in resistant hosts. On the basis of electron-microscopic pictures, Bernhard[13] supposes that most, if not all, strains of mice carry this agent. He relates tumor development to concentration of virus particles in cells, that is, to the ability of the host cells to support virus multiplication.

There are other observations, correlating tumor development and "masking" of causative virus to concentration of the virus. For example, when the original infective dose is small, the virus is often not recoverable from Rous sarcomas.[22]

Virus infectivity is much reduced in resistant hosts often to the point of appearing "masked." Turkeys can be infected with Rous sarcoma virus, but the viral content of turkey sarcomas is low, or absent.[66] There is a resemblance between Shope's "masked" rabbit papillomas and pseudo-rabies virus in cattle and certain strains of Newcastle disease virus in mice.[64]

The virus-containing rabbit papilloma is heterotransplantable, but when it turns into a carcinoma and loses its viral content[58] it also loses heterotransplantability. In the in-between stage, the virus is masked, its presence being demonstrable by serologic reactions only.

Neoplastic Transformations in Tissue Cultures.—Normal tissues grown in vitro frequently, and often rapidly, give rise to cells with chromosomal abnormalities which can be isolated and carried as distinct lines possessing features of malignant cells. The genetic interpretation is that a drastic environmental change goes with selection of "natural" mutants, or that such new types of cells are developed by some adaptative mechanism favoring proliferation of the mutant. The in vitro behavior can be regarded as a model for the in vivo behavior. In both, the normal genotype is at a selective disadvantage, the new type, at an advantage. In tissue cultures the forces of resistance to viruses, present in vivo, are lacking and thus latent viruses can become unmasked. Briefly, when a cell turns neoplastic in vitro it is possible that the media carried a virus or that a "masked" virus became unmasked or that normal cells mutated in the abnormal environment. When a "masked" virus becomes unmasked, it has the basic features of the original virus. Thus, the mouse parotid tumor virus, grown in the presence of monkey tissue, retains prime affinity for salivary glands of mice. When infectious monkey viruses are activated in vitro they will only infect monkeys.

When the range of a tumor virus is broadened in tissue culture as reported by Stewart and associates,[123,124] it is likely to be limited to cells of the original species. This effect may be due either to adaptation or to increase of concentration of the virus or to a modification of the virus, similar to that of transformation of a rough into a smooth type of micro-organism. Events noted with tumor viruses have not been thoroughly analyzed.

Poor Transplantability of Some Tumors.—Why are many spontaneous and chemically induced neoplasms poorly transplantable? Presently this question is specifically directed to viral tumors, such as those of the parotid gland of mice, to lymphomas and to chemically induced sarcomas of chickens. Factors which inhibit growth of nonviral tumors are: histoincompatibility (applicable to all tumors) and lack of conditioning factors (applicable to dependent tumors).

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The factor which specifically inhibits viral tumors is immune response to the virus. The main factor which promotes growth of grafted cells is lowering of defense mechanisms. Tumor viruses can promote cell proliferation to the extent of violation of the laws of genetics of transplantation. These forces have not been analyzed adequately in given situations to explain why the grafted tumors failed to grow.

Parotid tumors of mice are not readily transplantable even though they probably carry a virus and the host C3H strain is fairly well inbred. Of 63 chemically induced tumors, Peacock[97] succeeded in transplanting only 17 more than one passage. He concluded that chemically induced tumors are virus-free. However, spontaneous avian lymphomas which are believed to be virus-induced are even less readily transplantable.

The viral parotid tumors of mice are poorly transplantable by cells, and the recipients do not develop tumors in their own parotid glands.[24,80] Obviously, the virus is "masked" in cells and is not liberated from disintegrated or multiplying cells in the host, at least not in sufficient numbers to cause primary tumors in mice with grafted tumors, though it must be assumed that they carry the virus. The leukemic cells carrying the virus of Friend, on the contrary, apparantly fail to multiply at sites of the grafts but liberate the virus causing leukemic proliferation of cells at distant sites, notably the spleen.[90]

THOUGHTS FOR FUTURE RESEARCH

Every section of this review reveals numerous unanswered questions. Foremost among these are the problems related to "masking," the study of which is needed to understand the biologic behavior of viral tumors and to aid in search for viruses in tumors in general.

It is desirable that in experimental studies with animals the degree of homozygocity of the host strain be ascertained by cross-grafting of normal tissues, e.g., skin. If an apparently malignant tumor is not readily transplantable in the well-inbred strain of origin the possibility that it is virus-induced should be investigated. On the one hand, tumor viruses can enable a cell to violate histocompatibility factors (as that of rabbit papilloma); on the other hand they need not confer upon the cell ability of unrestrained growth (as parotid tumor virus of C3H mice). It should be recalled that embryos, the newborn, and cells in tissue cultures are not subject to the inhibitory forces present in the adult organism. A necessary step in tumor initiation by virus is the entry of virus into susceptible cells. Rous virus is ineffective by the intravenous route, unless some local lesion "attracts" the virus and enables its entry into (probably proliferating) fibroblasts. Once the tumor growth is well on its way, antibodies against both tumor cells and viruses are less effective, either because of antigen excess or, in case of viruses, because of the intracellular location of the virus. Means of suppressing antibody production may be helpful as is the use of hamsters, in which, for reasons unknown, growth of alien cells is less inhibited than in any other mammal studied. High-speed centrifugation may be helpful to concentrate virus, and immune reactions and electron micrographs to identify the presence of virus.

The ability of virus-infected benign tumors to be heterotransplantable is a possible lead to other heterotransplantable tumors being virus induced, as has been suggested by Greene.[58] Four human tumors are characterized by such behavior: the meningioma, the Schwannoma, the mixed salivary gland tumor, and the bladder papilloma.[59] Consequently, search for virus in heterotransplantable human tumors is worth intensifying. Of even greater promise is the introduction of presumed human viral tumors into tissue cultures of human or primate embryos.

To overcome complications arising from coexistence of virus with antibody, presumed virus obtained from benign and malignant tumors, could be concentrated and purified by known techniques,[23] tested for specific antigenicity with patients' sera and for replication by growth in embryonal tissue cultures.

In view of the presence in the plasma of leukemic chickens of large quantities of virus, it is desirable to renew attempts to electron micrograph pellets of high-speed sedimentable material from human leukemic plasma.

The likelihood of identifying in animals many more tumor viruses than presently known is great. The reasoning that fundamental biologic phenomena observed in animals are likely to be true for man is sound. The opinion that most natural tumors in mammals, even though they are *transmissible* by virus-like agents, are not likely to be *caused* by them should in no way discourage search for viral tumors in man and animals. Identification of virus-like agents in human neoplasms, whatever their nature, is a great challenge to cancer research.

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SUMMARY

1. There is overwhelming evidence that viruses (submicroscopic replicating agents) can induce tumors in many species of animals and that in one (fowl) most or all mesenchymal tumors are produced by viruses.

2. In general, tumor viruses resemble classical viruses. They can exist in a variety of sub-types closely related immunologically and cytopathogenetically, yet exhibiting individual features, as the family of leukosis and sarcoma viruses in chickens. They appear adaptable and can probably mutate.

3. There is strong evidence indicating that "tumor viruses" vary widely in character, one extreme being akin to pathogenic micro-organisms capable of *producing* a neoplasm under natural conditions; the other extreme being genes (DNA) which can *reproduce* a neoplasm by a process analagous to transduction. Accordingly, the pathogenesis of virus tumors is diverse.

4. It is probable that the principles disclosed in studies of animal tumors apply to man and that techniques already available may disclose the existence of similar agents in many human tumors.

5. However, it also seems probable that most of these agents may prove to be of the type which "reproduce" rather than "produce" mammalian neoplasms. It follows that from the standpoint of prevention and control at least, carcinogenic chemicals, hormones, and radiations are to be considered as prime causative agents of most neoplasms. Nevertheless, the spectrum of replicating agents which may cause neoplasms in man, the nature of these agents and their role in tumorigenesis, deserve intensified research; for this is the frontier of knowledge,

with promise of more exciting, though conceivably less practical, contributions to cancer control than that of the other known classes of carcinogens.

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RADIATION-INDUCED LEUKEMIA IN MAN, WITH PARTICULAR REFERENCE TO THE DOSE-RESPONSE RELATIONSHIP

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IT IS now accepted that exposure to nuclear and allied radiations can cause leukemia in man. The evidence for this is derived from a number of sources, particularly from studies of the survivors of the atomic explosions over Hiroshima and Nagasaki,[1-5] from an analysis of the incidence of leukemia in over 13,000 patients treated with x-rays for ankylosing spondylitis,[6-11] and from a study of the frequency of past exposure to x-rays, for unassociated reasons, in patients developing leukemia.[12,13] Additional data have been obtained from investigations of the incidence of leukemia in children irradiated during infancy for suspected thymic enlargement,[14,15] from a study of children exposed in utero during the course of x-ray examination of the maternal abdomen and pelvis,[16] and from studies of the causes of death of American radiologists.[17-22]

There is agreement that the major increase occurs in the acute forms of leukemia, particularly in acute myeloid leukemia. There is evidence also of an increase in chronic myeloid leukemia, but the data do not suggest that the incidence of chronic lymphatic leukemia is raised. No increase in this form of the disease was found among the Japanese survivors; the natural incidence, however, of chronic lymphatic leukemia in Japan is low, and it was not until studies of irradiated Europeans also failed to demonstrate an increase incidence that it could be held with some confidence that radiation exposure does not appear to induce chronic lymphatic leukemia in man.

The recognition of the leukemogenic nature of radiations toward man immediately poses the question of what is the form of the relationship between the incidence of leukemia and the radiation dose, and, in particular, whether there exists a threshold dose below which no increased probability is incurred of developing leukemia. Germane to the study of the dose-response relationship is the question of what is the average length of the latent period between exposure to radiation and the clinical development of the disease. These problems will now be considered in some detail with particular reference to the leukemia incidence among the Japanese survivors and that among patients given x-ray therapy for ankylosing spondylitis.

TABLE I. THE INCIDENCE OF LEUKEMIA AMONG SURVIVORS OF THE HIROSHIMA ATOMIC BOMB EXPLOSION

DISTANCE FROM HYPOCENTER AT TIME OF EXPLOSION (meters)	NUMBER SURVIVORS ON JAN. 10, 1950*	NUMBER CASES OF LEUKEMIA				INCIDENCE PER 10,000 PERSONS (TOTAL CASES)	NUMBER EXPECTED DEATHS, AMONG SURVIVORS IN AN 8-YEAR PERIOD 1947-1954‡	RATIO OF CASES OBSERVED (1947-1954) TO EXPECTED
		CONFIRMED		SUSPECTED				
		ALL YEARS	1947-1954	ALL YEARS	1947-1954			
Less than 1,000	1,250	16	15	0	0	128.0	0.15	100.0: 1
1,000–1,499	10,350	28	28	1	1	28.0	1.32	22.0: 1
1,500–1,999	18,450	6	6	1	1	3.8	2.33	2.6: 1
2,000–2,999	30,350	7	6	0	0	2.3	3.96	1.5: 1
3,000 or more	37,700	4	4	2	2	1.6	4.83	1.2: 1
All distances	98,100	61	59†	4	4	6.6	12.59	4.7: 1

*Rounded off to the nearest 50.

†Two cases are omitted in comparison with the "All Years" group. The onset of symptoms in one was in 1955, and in another, dying in 1955, the date of onset is unknown.

‡Calculated from Japanese mortality data for 1952.[24] The figures must be regarded as approximate estimates.

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THE INCIDENCE OF LEUKEMIA AMONG THE JAPANESE SURVIVORS

The data in Table I are based on information provided by the Atomic Bomb Casualty Commission to the Medical Research Council, and originally published in the Medical Research Council's Report on the Hazards to Man of Nuclear and Allied Radiations.[23] It will be seen that the incidence of leukemia per 10,000 survivors, up to the end of 1954, steadily diminishes the more distant the survivors were from the hypocenter at the time of their exposure. For each zone calculations have been made of the expected incidence of leukemia, up to the end of 1954, from the Japanese national mortality statistics.[24] Comparisons with the observed numbers of confirmed cases of leukemia show that the ratio of observed to expected cases falls from 100:1 among survivors from the 0-1,000 meter zone to 1.2:1 among those 3,000 meters or more from the hypocenter.

The behavior of this ratio is a clear indication of some form of dose-response relationship. The question of how much dose was, in fact, received by each individual survivor is controversial. In particular, the components of the dose due to fast neutron irradiation and to local fall-out are difficult to estimate. Hempelmann[25] stated, in 1957, that the latest unclassified estimate for the mean dose to the survivors from 1,500-1,999 meter zone was about 50 rem. By the end of 1954, 6 confirmed cases had been found in this zone, and 1 suspected case. The expected number was 2.3, and the difference between the observed and expected numbers is on the border line of significance ($P = 0.03$). Further cases occurring among this group in the years subsequent to 1954 could well enhance the significance of this difference. It might be argued, therefore, from this data that if a threshold dose exists, it lies below 50 rem.

It is pertinent at this stage to discuss how freely data based on the Japanese survivors can be transposed to the consideration of American and European populations. A comparison between the national mortality data of Japan[24] and those of England and Wales[26] is instructive. The most striking difference is that the Japanese age-specific death rates for leukemia show virtually no increase with advancing age, whereas the increase is marked in England and Wales (Table II). There appears little doubt, also, that chronic lymphatic leukemia is a rare condition in Japan, whereas it is a common form of leukemia in England and Wales. The rising death rate, however, in England and Wales among the older age groups is clearly not solely explained by an increase in lymphatic leukemia (Table III); on the other hand, though no data are available, it would be surprising if the absence of any great increase in the death rate with increasing age among the Japanese is solely due to the rarity of chronic lymphatic leukemia.

When comparisons are made between the lengths of the latent periods in the Japanese survivors developing leukemia and those in the spondylitis cases developing leukemia following a single course of treatment, a further difference is evident. In the Japanese cases the latent period has been measured from exposure in August, 1945, to the time of the development of their symptomatology, and in the spondylitis cases from the first course of treatment to the clinical recognition of the disease. From 1948 to 1954, inclusively, there is little convincing evidence of a fall in the annual incidence of leukemia in the Japanese

TABLE II. AGE-SPECIFIC DEATH RATES FOR LEUKEMIA: ENGLAND AND WALES AND JAPAN (1952—BOTH SEXES)

AGE GROUPS (YEARS)	DEATHS PER MILLION LIVING PERSONS	
	ENGLAND AND WALES	JAPAN
0–4	51.1	26.8
5–9	32.4	14.7
10–14	22.6	15.6
15–24	19.8	13.5
25–34	17.2	12.8
35–44	26.5	17.3
45–54	40.9	18.3
55–64	81.0	18.3
65–74	128.0	15.1
75+	149.6	9.3
All ages	46.3	16.6

TABLE III. AGE-SPECIFIC DEATH RATES—MYELOID AND LYMPHATIC LEUKEMIA ENGLAND AND WALES—1952

AGE GROUPS (YEARS)	DEATHS PER MILLION LIVING PERSONS	
	MYELOID	LYMPHATIC
0–4	8.0	23.1
5–9	5.8	14.1
10–14	10.9	8.1
15–24	9.8	5.1
25–34	8.8	4.4
35–44	16.7	5.5
45–54	23.5	9.6
55–64	39.2	30.4
65–74	55.5	55.8
75+	64.9	62.4
All ages	20.7	16.5

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TABLE IV. THE INCIDENCE OF LEUKEMIA IN DIFFERENT YEARS AMONG SURVIVORS OF THE HIROSHIMA AND NAGASAKI ATOMIC BOMB EXPLOSIONS

YEARS OF ONSET	HIROSHIMA*	NAGASAKI*	INCIDENCE PER 10,000 PERSONS		
			HIROSHIMA	NAGASAKI	HIROSHIMA AND NAGASAKI
1946	0	1	0.0	0.1	0.05
1947	3	2	0.3	0.2	0.3
1948	10	3	1.0	0.3	0.7
1949	5	3	0.5	0.3	0.4
1950	7	6	0.7	0.6	0.7
1951	11	10	1.1	1.0	1.1
1952	11	8	1.1	0.8	1.0
1953	10	3	1.0	0.3	0.7
1954	6	7	0.6	0.7	0.7
All years	63	43	6.6	4.5	5.6

*Both confirmed and suspected cases.

TABLE V. THE RELATIVE FREQUENCY OF DIFFERENT LATENT PERIODS IN LEUKEMIA FOLLOWING ONE COURSE OF TREATMENT

(Court-Brown and Doll[8])

LATENT PERIOD (MONTHS)	MORBIDITY RATE* PER 10,000 MAN-YEARS
0–	0
24–	4.0
36–	10.3
48–	13.4
60+	0

LATENT PERIOD (MONTHS)	NO. OF CASES OF LEUKEMIA
The Distribution of Latent Periods—Including More Recent Cases of Leukemia	
0–	0
24–	2
36–	5
48–	6
60+	0

*Based on 10 cases of leukemia.

survivor population (Table IV). The fluctuations are probably largely random in view of the small numbers of cases in any one year. Furthermore, considering the way in which the latent period was measured, it is likely that some cases were diagnosed clinically, in 1955, which had developed symptoms in 1954. In contrast, in the spondylitis cases developing leukemia following a single course of treatment, there is every evidence of a peak period during the fourth and fifth years after exposure. Additional cases that have occurred, since 1955, have lent further emphasis to this finding (Table V). Only one case has been found in which the latent period exceeded 5 years. This case, the only female patient noted in whom the disease followed a single course of treatment, was not one of the survey cases; the latent period was 8 years.

TABLE VI. THE INCIDENCE OF LEUKEMIA AND THE AGE OF THE PATIENT AT THE TIME OF EXPOSURE

AGE (YEARS)	STANDARDIZED INCIDENCE RATES, PER 1,000 PATIENTS (BOTH SEXES)	THE AVERAGE AMOUNT OF RADIATION GIVEN TO MEN OF DIFFERENT AGES (MEAN SPINAL MARROW DOSE)
14–	1.1	858 r
25–	2.6	894 r
35–	2.4	840 r
45–	5.2	776 r
55+	5.6	576 r

A second interesting difference is that the incidence of leukemia in the spondylitis cases rises quite clearly with age, in spite of a distinct tendency to give the older cases less x-ray treatment (Table VI). A similar rise was not found among the Japanese survivors.

These differences between on one hand, the Japanese data and, on the other hand, the English and Welsh mortality data and also the spondylitic cases suggest that there are racial and environmental differences to be taken into account. It is not possible to ascribe even part of the differences to any peculiarity inherent in the spondylitis cases, as evidence gathered by Faber in Denmark further strengthens the idea, as far as the latent period is concerned, that the Japanese and Europeans react differently. Faber found that in comparison with controls there was a significantly increased history of radiation exposure for medical purposes among cases of acute leukemia. This difference only existed for exposure within 5 years of the development of leukemia, although for chronic myeloid leukemia his data suggest that the latent period can exceed 5 years. This finding supports that of the spondylitis cases and contrasts with the evidence from the Japanese survivors.

It seems, therefore, reasonable to sound a note of caution about extrapolating too freely from the Japanese data to American or European populations.

THE INCIDENCE OF LEUKEMIA AMONG CASES OF ANKYLOSING SPONDYLITIS TREATED WITH X-RAYS

The study of the incidence of leukemia in patients treated with x-rays for ankylosing spondylitis was designed to provide information on the dose-

response relationship for radiation leukemogenesis. While it was realized that direct information could not be obtained on the effects of doses of x-rays as low as those employed in diagnostic radiology, it was hoped that sufficiently reliable data would be obtained on the effects of high doses to permit confident extrapolation to the possible effects of low doses. In this study the dose-response relationship was based on the examination of the records of 11,287 male patients among whom 37 cases of leukemia were found. As x-ray treatment to the spinal axis is by far the most common form of treatment for this disease, considerable efforts were made to study the distribution of dose in the spinal bone marrow. Eighteen of the patients developed leukemia following treatment solely confined to the spinal axis.

It is worth while considering in some detail the methods used in this survey to examine the radiation dose in relation to the incidence of leukemia, as the problems involved are difficult to solve and are of general interest to all who may enter this field of study.

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The choice of the parameter of dose to study is of necessity influenced by what views are held on the problem of the presence or absence of a threshold dose. If a threshold is thought to exist, then the correct parameter of dose to measure is the maximum dose at a point within the marrow. In fact, the threshold dose will be the maximum dose which just falls short of increasing the probability of any given individual developing leukemia. If a threshold dose exists, then, by implication, some recovery with time can occur from the initial damage to the marrow which ultimately develops into the leukemic state. If there is no threshold dose, then the more suitable parameter of dose is the mean marrow dose, and, by implication, there is no recovery from the original damage. Finally, whichever postulate is used, it would appear desirable to introduce some weighting, for the chances of the development of leukemia in each postexposure year.

In the survey of the spondylitis patients three different forms of dose were studied, the total body energy absorption (the integral dose), the maximum dose at a point in the spinal marrow, and the mean dose to the spinal marrow. The first parameter will not be discussed further, as it can be of value only if it bears some relationship to the bone marrow integral dose. This is likely to be the case under the conditions of uniform, or almost uniform whole-body irradiation, but unlikely to be so with varying types of partial-body irradiation. When the incidence of leukemia was related to the maximum dose in the spinal marrow the results provided no evidence for the existence of a threshold dose (Table VII, Column A). In constructing this relationship, however, no weighting was introduced for the recovery factor, as no evidence exists, as to what weighting should be given. Also, with the exception of the first postexposure year,* an individual was considered equally at risk to the clinical development of leukemia in each year subsequent to exposure. This assumption was made as, at the time of the survey, it was felt that perhaps greater reliance should be given to the Japanese data on the latent period in view of the greater number of cases of leukemia.

*This year was given zero weighting as no case was found in which the clinical leukemic state was recognized in the first year after exposure.

It has already been pointed out, however, that a different view is now held and that there may be racial and environmental differences to be taken into account. A further problem that arises in studying the effect of the maximum dose at a point in the marrow is the necessity, where less than whole-body irradiation is employed, of studying only cases in which comparable volumes of marrow are irradiated. This was not possible in the spondylitis cases in view of the small number of cases of leukemia, and it is felt that this failure may have introduced a systematic bias into the results based on the maximum spinal marrow dose.

TABLE VII. ANKYLOSING SPONDYLITIS. THE RELATION BETWEEN THE ANNUAL CRUDE INCIDENCE OF LEUKEMIA PER 10,000 MEN AND THE SPINAL MARROW DOSE

A		B		C*	
MAXIMUM DOSE AT A POINT IN THE SPINAL MARROW, WHOLE GROUP (r)	INCIDENCE	MEAN SPINAL MARROW DOSE, WHOLE GROUP (r)	INCIDENCE	MEAN SPINAL MARROW DOSE, SPINAL CASES ONLY (r)	INCIDENCE
0	0.49	0	0.49	0	0.49
less than 500	2.2	less than 500	2.16	less than 500	1.53
500–999	4.1	500–999	4.59	500–999	4.72
1,000–1,499	4.2	1,000–1,499	6.99	1,000–1,249	6.75
1,500–1,999	11.3	1,500–2,249	12.18	1,250+	8.12
2,000–2,749	13.0	2,250+	63.65		
2,750+	17.6				

*For details of the variation in incidence depending on variations in groupings of dose and for standardized incidence rates, see Court-Brown and Doll.[8]

When the incidence of leukemia was related to the mean spinal dose again no evidence was obtained for the existence of a threshold. As in the case of the maximum dose at a point in the spinal marrow the individual was considered equally at risk in each postexposure year with the exception of the first. As there is by implication no recovery factor, no additional weighting is necessary for this. The data shown in Table VII (Column B) refer to the whole study population. In these there were a number of cases in which extraspinal sites, such as the hip joints had been irradiated. For these cases, as no account was taken of the dose to the extraspinal marrow, the true mean marrow dose is not known. Consequently, it was felt desirable to study the dose-response relationship for those cases given spinal irradiation alone (Table VII Column C). The most satisfying relationship is a linear one, and the equation to the line is $y = 0.00520x + 0.49$, where $y =$ the annual incidence of leukemia per 10,000 men, and $x =$ the mean spinal marrow dose in roentgens. From this equation it can be deduced that a mean spinal marrow dose of 94 r will increase the annual

incidence of leukemia by 0.49 cases per 10,000 men, i.e., will double the incidence. If it is assumed that no threshold exists and that between one-third and one-half of the total red marrow is in the spinal column, then the mean dose to the whole body marrow which will double the incidence of leukemia will lie between 35 and 50 r. In other words, the probability of developing leukemia per year lies between 1×10^{-6} and 2×10^{-6} per r averaged over the whole red marrow system.

In constructing the dose-response relationship for the cases given only spinal irradiation, the point for zero therapeutic dose (0.49 cases of leukemia per 10,000 men per year) was considered as reliable as the other points. It was derived from the British national mortality statistics and was adjusted to allow for errors in death certification and for the living cases of leukemia. It is, of course, based on the assumption that the incidence of leukemia among cases of spondylitis not treated with x-rays, is the same as in the general population of the same sex and age distribution. Investigations have shown no reason to doubt the validity of this assumption. Recently criticism has been raised against the inclusion of the point for zero therapeutic dose in the calculation of the regression line.[27] If this point is left out, the equation to the line becomes $y = 0.00634x - 0.34$.[11] Calculation of the probable limits of the regression line indicate that, if all the cases of leukemia are radiation-induced, the value for the threshold may be either zero or lie between zero and 400 r mean spinal marrow dose. If, however, the threshold has a value greater than zero, then it cannot be expressed in terms of a mean spinal dose, which by definition is a dose parameter suitable only for measuring a nonthreshold response. It follows, therefore, that if the dose-response relationship for leukemia in an irradiated population gives evidence of a threshold using the mean marrow dose, re-examination of the population using the maximum dose at a point in the marrow will be necessary to ascertain the probable magnitude of the threshold.

On the basis of their findings in the survey of irradiated cases of ankylosing spondylitis, Court-Brown and Doll have suggested, as a working hypothesis, that the induction of leukemia in man by ionizing radiations is a nonthreshold event. It is to be emphasized that this is only a working hypothesis, which will permit the design of further studies to prove or disprove its validity.

CONCLUSIONS

There is little doubt that the application of epidemiologic techniques to the study of human-delayed radiation effects, such as leukemia, may well prove of inestimable value. It is essential if the values of maximum permissible levels of exposure are to be correctly assessed, and only in this way may information be gained about the existence, or lack of existence, of dose thresholds. Furthermore, beyond the immediate objectives of assessing the risk of radiation exposure, the information may be of considerable value in gaining a better insight into the fundamental features of malignant disease.

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THE IMMUNOLOGIC THEORY OF CANCER. SOME IMPLICATIONS IN HUMAN PATHOLOGY

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IN 1954, I put forward a new theory of the nature of the cancer cell which has since been elaborated and expanded considerably.[10-14] It is not possible to summarize these publications here, for they are themselves mainly summaries over a wide field, including some of the many experiments made in my laboratories. To provide a background to the present observations, the publications[11-13] should be consulted; most of the relevant references will be found there. Shorn of all technicalities, the theory states that the neoplastic cell is a deficient cell lacking one or more of those factors (tissue-specific antigens) which give it identity as a tissue. The more it lacks tissue identity, the greater its malignancy. If it further lacks individual identity (isoantigen), which is more a feature of easily transplantable tumors, the greater this lack, the greater is its capacity for growth in different strains.

Two points must be emphasized. First, it was the discovery of an increased isoantigenic immunity induced by tumor-inhibiting carcinogens and some related noncarcinogens which first led us to the concept of antigenic change as synonymous with the initiation stage of carcinogenesis. Isoantigenic change is, however, in our view not an essential, nor even necessary, feature of malignancy, but it provides a pointer to tissue-specific antigenic change, followed later by loss, which we consider is the all-important factor in chemical carcinogenesis. It is, in fact, possible that the initial isoantigenic change may tend to inhibit carcinogenesis by evoking antibodies which attack the nucleus and are thus lethal to the cell. Second, the term antigen lack covers also lack of antigenicity. Theoretically, this latter state could be brought about not only by loss, but by quantitative alterations in different antigens within the cell. The main evidence strongly favors the view that actual loss of cytoplasmic antigen occurs but in experiments on tumor enhancement,[13,16] which may be regarded as a state of increased malignancy, there was some evidence of relative gain in one cytoplasmic antigen. This probably was associated with actual deficiency in another, but the possibility remains that the deficiency is relative and not absolute. There is indeed evidence[3] that in transplantation immunity the more wide-ranging the strain adaptibility of the tumor, the greater is its isoantigenic content. In this case it is assumed that tumor isoantigen neutralizes circulating antibody before it

reaches the tumor cell. There is, however, no obvious reason why isoantigenic gain and tissue-specific antigen loss should not occur in the same cell.

We have previously reviewed the direct evidence for loss, and further evidence has been given by Kay[18] in showing that the human bladder cancer cell fails to exhibit "blood group" A and B antigens, the deficiency increasing with the degree of malignancy. He thinks that the antigens are not missing but unavailable owing to cell membrane alterations, but this seems to us unlikely. It is more likely that the apparent lack of these blood group antigens is real and indicates a loss of some essential part of their chemical structure. According to my theory, this lack of "blood group" antigens would not itself be the cause of the neoplastic state, for they are obviously not tissue-specific antigens but widely distributed ones. Their deficiency may, however, reflect a loss of a similar substance(s) normally incorporated in the tissue-specific antigen(s) which may well prove to be a lipoprotein complex.

Unitarian theories are often suspect for their authors, particularly medical ones, are apt to be subjects of megalomania. Yet in the cancer field there is every justification for the attempt, for about no other disease has such a mass of facts accumulated, facts completely beyond the capacity of any one man to assimilate. If for no other reason, the wide range of scientific disciplines embraced and the application of every advance in them to cancer studies, makes it quite impossible to comprehend them all. A unifying theory has, therefore, the merit of inducing scientists, pathologists, and clinicians to see whether their own particular facts can be reconciled with it. If a theory finally emerges which can be reconciled with this array of facts, then there will be every justification for a concerted effort to test its experimental validity. Until the nature of cancer is known, there is little hope of doing anything radical about it. The failure of empirical methods of treatment and the minor success of methods based on experimentally discovered properties of the cancer cell point strongly to this conclusion.

In this review, I am not directly concerned with the older experimental facts on which the theory is based, nor the new ones which continue to support it, but with its bearing on the pathology of human tumors. How far can this pathology be reconciled with the theory? Even on this narrower basis there is only space to consider a few representative types. These have been mainly chosen to illustrate various aspects of the immunologic theory.

CHEMICAL CARCINOGENESIS

In the proportionally few human cancers known to be due to definite chemical carcinogens the mechanism of induction according to theory is briefly as follows. The carcinogen binds with protein complexes in the target cell of which one or more are tissue identity proteins. Autoantigenic complexes are formed, and several possibilities arise. The cell may be destroyed by antibody particularly if the nuclear isoantigens are much involved. Cells, if any, which escape this attack may contain antigenically modified cytoplasmic (or membranous) identity proteins which, there are reasons for thinking, may not elicit lethal antibodies but surface antibodies possibly protecting them from further nuclear

assault by a blocking action. Cells exposed for a brief period may leave no modified descendants, but after a quite short period some permanent and inheritable modification occurs. If exposure to the carcinogen continues, cell proliferation is induced, possibly as a by-product of the local immune reaction. The antigenic stimulus progressively increases, and the resulting antibody attack on the cytoplasmic antigens results in a progressive adaptation involving deletion of the antigen(s) concerned. If the carcinogenic stimulus stops, a focus of modified cells is left which is capable of being promoted to the malignant state at any time in the future, either by a carcinogen or by any agent capable of inducing cell proliferation of a specialized kind. If the exposure to carcinogen continues, antigenic deletion gradually approaches the stage which renders the cell capable of invasive growth. But the same, though much slower, process can be brought about by a few of the many agents known to stimulate cell division. To explain this, it could be assumed that such promoting agents are really weak carcinogens, for which there is some evidence. Or alternatively they might interfere with the nutritional state of the tissue as well as stimulating cell division, thus rendering the already antigen-deficient cells more likely to undergo further antigenic loss. That the carcinogenically modified cells, after exposure has ceased, continue to exert an antigenic effect by retention of modified self-replicating antigen does not seem likely. If they did, they would be expected to become neoplastic without further stimulus at some later, albeit much later, stage. Moreover, any agent stimulating mitosis should be an effective promoting agent if that were the case, and this is not so.

As we shall see, it seems likely that promoting agents are of great importance in the genesis of human cancer possibly determining the appearance of tumors in tissues such as the bronchus and cervix which have previously been exposed to a carcinogenic stimulus. Such an event can theoretically happen at any age, but in man it is apt to occur in his later years when hormonal imbalance and other causes of pathologic overgrowth have had time to produce pathologic changes in the supporting tissues. It is well known that a relatively brief exposure to a chemical carcinogen can be followed by a malignant growth many years later. The bladder cancer of the dye worker exposed to 2-naphthylamine is one example.

What clinical evidence is there that chemical carcinogens may act as haptenes and thus induce an antigenic change in the tissues? Some tars and many of the aromatic amines like aniline, benzidine, naphthylamine are capable of causing allergic reactions in man such as dermatitis, rhinitis, etc. They must therefore bind with tissue protein in vivo as they, along with polycyclic carcinogens from coal tar, can be made to do in vitro. The carcinogens among these compounds must in theory be assumed to bind in a specific way to an identity protein complex. Inorganic elements such as nickel, chromium, and arsenic are all both potential allergens and carcinogens and presumably therefore modify the antigenic structure of the proteins with which they react.

IONIZING RADIATIONS

What of potentially carcinogenic radiations like x- and ultraviolet rays? The skin may become hypersensitive to both. From experimental work I have

postulated that ionizing radiations may be able to induce changes in protein structure and thus produce autoantigens. Radiation sickness, which resembles serum sickness in some ways may be an expression of the immune reaction thus induced. Although long recognized in animals by research workers the carcinogenic effects of ionizing radiations in man are only now being appreciated. In experimental work there are many examples but in man, though many skin carcinomas were observed in the early x-ray days from overexposure, the thyroid and bone marrow are as yet the only tissues definitely known to be made more prone to neoplastic change by previous irradiation. No doubt many other examples will follow.

PROLONGED HYPERPLASIA

Cancer in man is probably not usually due to the continuous action of a carcinogen but much more often to a period of exposure followed, often at a much later period, by a promoting stage, usually hormonal or inflammatory in character. As we shall see, even in the case of bronchial cancer, this seems to be the more likely sequence of events. One of the big questions is whether excessive long-continued hyperplasia alone may induce cancer in man. Consider the well-known instances where it plays a dominant part. In many of these the hyperplastic stimulus is hormonal, and tumors of the breast, uterus, ovary, prostate, and thyroid are probably all intimately linked up with such excessive stimuli. Cystic hyperplasia is a forerunner of carcinoma of the breast and endometrium, both of which are probably induced by excessive or too prolonged estrogen stimulation. Prostatic enlargement may prove to be due to prolonged estrogen stimulation. It is most important to note, however, that continuous hyperplasia alone does not in itself promote the cancerous change. The milking cow does not develop tumors of the breast, nor does the typical case of hyperthyroidism develop cancer of the thyroid. It is only when some pathologic change associated with, or due to, hyperplasia occurs that the precancerous state arises. Such a change would appear to be the result of hyperplasia without complete function. The thyroid hyperplasia brought about by iodine deficiency is hyperplasia without normal function, so is cystic hyperplasia of the breast, and possibly similar factors operate in the senile prostate. The cell is stimulated, but its normal secretions are not produced. Neither thyroxine nor milk is secreted, the cell lacking iodine in the case of the thyroid and other hormones (e.g., progesterone, prolactin) in the stimulated nonlactating breast. In such deficiency states, excessive cell proliferation leads to premature cell death (so-called involution) with reactive changes in the stroma resulting finally in such conditions as nodular goiter or cystic hyperplasia. It is such changes coupled with prolonged excess hormone stimulation that are probably the real basis of the precancerous state. Long-continued stimulation without adequate function are common factors preceding the outgrowth of human cancers. Under these conditions (i.e., of scarring and widespread stromal and vascular changes) the nutritional state is impaired locally, and yet the tissue may continue to be subject to the same hyperplastic stimuli which have brought it to this state. Such conditions must tend to promote a local deficiency state, which I have suggested has an analogy in the conditions of tissue culture. If cells are present lacking some identity protein as a result of a previous carcinogenic stimulus, they should tend to be selected, as their nutritional requirements are less and hence their chances of survival greater. Or, even if there was no previous carcinogenic

stimulus it is not difficult to imagine deficient cells arising under these conditions. Cytoplasmic identity proteins controlling functions peculiar to that cell are likely to be among the most structurally complex elements in the cell. A local deficiency state would in theory tend to encourage the formation of cells deficient in identity proteins, and such cells would have a greater potentiality for survival. With the gradual deterioration of local conditions, cells with lower and lower identity protein levels will tend to be selected until finally the degree of loss is incompatible with recognition by the normal growth-regulating mechanisms. The neoplastic cell then emerges, and its properties could be determined by this loss in ways I have previously explained in the writings cited. This is theory, but from a practical standpoint it must be stressed that malignant transformation in tissue culture may well prove to be an exact repetition (in a nutritional sense) of the neoplastic process following long-continued hyperplasia in vivo. I believe that it will be found that transformation of normal to neoplastic cells in tissue culture is a common event and may even be essential for survival. Identity proteins are, of course, not required under these conditions, and their loss would greatly increase the chances of survival of the cell in tissue culture. Hence, possibly the ease of culture of malignant cells as compared with their normal prototypes, at any rate until the latter have become transformed into a state which may prove to be a gradual progression toward neoplasia.

Probably very many human cancers in organs particularly subject to intense stimulation arise in the way suggested. The theory does not in any way preclude a role for chemical carcinogenesis as well. It may well be that exposure often occurs in man and thus renders the already modified tissue more likely to undergo malignant change when submitted to abnormal hormonal stimulation. The pathogenesis of breast, thyroid, ovarian, endometrial, and prostatic cancers may all be on similar lines. On the other hand the evidence suggests that both cancer of the cervix and of the penis are due to exposure to a carcinogen contained in retained smegma followed by promoting agents such as trauma and possibly estrogens in the cervix and chronic inflammation in the penis. Circumcision is completely effective in preventing cancer only if performed in early life, possibly before 5 years of age. In theory, therefore, cells already deficient in identity proteins must exist at an early age, and these are goaded into cancerous activity only if the hyperplastic and scarring conditions of chronic inflammation occur much later in life.

LIVER AND BONE CANCER

Of other types of excessive hyperplasia prominently related to human cancer, those of the liver and bone stand out. In the liver, again simple hyperplasia is not enough, for there must be in addition a damaging agent or nutritional deficiency producing reactive changes with ultimate scarring. As in other sites, it is also essential that the hyperplastic stimulus continues after the stromal changes have occurred. A precancerous state thus arises in portal cirrhosis whatever its cause, and it continues as long as the original stimulus, be it protein lack or a noxious agent, continues to act. At any time now a malignant hepatoma lacking in theory, one or more specific liver antigen(s) may arise and does so even in very young adults in races subjected to protein deficiency from early life.

If the liver-damaging factor ceases to be operative (e.g., after infectious hepatitis) the liver is not prone to cancer. In portal cirrhosis there is again hyperplasia without complete function, the ideal conditions for neoplastic change.

In bone, the high susceptibility of Paget's disease to sarcomatous change is, from an immunologic standpoint, most interesting. Here, possibly because of some unknown local deficiency, there is intense osteoblastic activity, and reactive stromal changes with fibrosis, and ultimately in some osteosarcomas multifocal in origin, emerge. Again a similar background of excessive proliferation in a progressively poorer nutritional environment resulting in theory in a strain of osteoblasts lacking in some degree their specific bone cell cytoplasmic antigens. The osteosarcoma of the young person may have an essentially similar origin, but here we know little of the precancerous state of the part of the metaphysis first involved. Perhaps it is significant that this is one of the few tumors where the role of trauma has remained debatable. It may be that a small focus of rapidly proliferating osteoblasts in an area of injury repair mimics temporarily on very rare occasions the conditions described in Paget's disease.

One may therefore regard many cancers of the breast, body of the uterus, prostate, and endocrine glands generally as the result of focal or general hyperplasia in malfunctioning tissue with ultimate failure to synthesize organ-specific antigens. Those of bone and liver can be included in this category, but not entirely, for cancer of the bone can be induced by radioactive substances, and cancer of the liver possibly by certain azo dyes, aromatic amines, or Senecio alkaloids which, for various reasons, may be included in the diet. On the other hand carcinomas of the skin and of the respiratory, alimentary, and urinary tracts must first be suspected of being caused by a chemical carcinogen if for no other reason than that repeated exposure is so much more possible in these sites. We have numerous examples in man: skin cancer due to coal tar and its high boiling point derivatives, mineral oils, and excessive ultraviolet light; bladder cancer due to aromatic amines and possibly bronchial cancer due to cigarette tar. I hold that the mode of action of such chemical carcinogens lies in a peculiar affinity for certain cytoplasmic lipoproteins, but the mode of action of an agent like ultraviolet light is still not obvious. It may be that overexposure induces the formation of a chemical carcinogen in the skin. In this respect it may resemble the greater susceptibility of burned tissue to cancerous change. Or chronic inflammatory changes in the dermis with overlying hyperplastic and keratotic epithelium may both be an expression of continued epithelial stimulation in a malfunctioning tissue.

ALIMENTARY TRACT

In the alimentary tract, apart from the mouth, we know little of the origin of the majority of tumors. Leukoplakia of the tongue is obviously a condition where epithelial cells are being stimulated to divide by external agents in a progressively worsening subepithelial environment, so that if the tongue epithelium is to survive it can only do so by becoming neoplastic. This teleologic phrase in no way clashes with the immunologic concept of neoplasia but merely emphasizes that cancer is a biologic escape mechanism allowing of cell survival in a region where its existence is threatened.

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STOMACH CANCER

In the human stomach we arrive, however, at a major mystery, with its proneness to cancer as contrasted with the virtual absence of stomach cancer in animals. Nor can it be experimentally produced, as I can well testify having, with Dr. Kennedy, tried the most diverse methods and agents (1949-1953), though a claim has been made that it can be induced in rats by the injection of 4-nitrostilbene.[2] In contrast, stomach cancer can be quite easily induced by the intramuscular injection of minced mucosa impregnated with methylcholanthrene. These facts, it seems to me, are important in the light of the immunologic theory. Clinical evidence has, for many years, indicated that gastric cancer is accompanied by achlorhydria and achylia. Some hold that these secretory faults precede and are essential to the development of cancer, and this idea is gaining further support from the reported high incidence of gastric cancer in pernicious anemia patients. Let us therefore assume that cancer cannot occur in a normally secreting stomach. If so, cells which have been initiated by exposure to a carcinogen must be destroyed by the gastric juice. Normal mucosal cells would not of course be affected, but those with a carcinogen-induced antigenic modification would then be regarded as "foreign" and destroyed as with any other "foreign" meat offered to the stomach. Such a fine discrimination might not operate in minced mucosa away from its normal environment. It should be pointed out here that even the small antigenic difference in the stomach of Wistar rats is sufficient to render their mucosa capable of damage after exposure to the gastric juice of a "foreign" rat.[1]

If this explanation proves correct, it would add very strong support to the immunologic theory which can already offer an explanation for many otherwise puzzling features of human cancer (e.g., anemia, weight loss, remote symptoms, dormancy). It is for such reasons that the theory commands support even in the absence of definite proof. The facts essential for proof may exist already, but alternative explanations of these facts are possible, and the use of theory to predict new facts is at present the most promising approach. It can be predicted from theory that only a faulty or nonsecreting mucosa can develop cancer. If this can be experimentally proved, the theory, though not necessarily true, is useful. It would not of course follow from this that gastric cancer is due entirely to chemical carcinogens. Achylia gastrica is the result of atrophic gastritis which is one indication that the stomach receives a large allotment of nonspecific insults resulting in damage and reparative hyperplasia. The relative infrequency of the ulcer-cancer sequence and the possibility that it does not even occur, suggests that chemical carcinogens, be they from heated fats or some of the many organic chemicals we ingest so widely, together with an inherited tendency to achylia, are the important factors in human stomach cancer. The inborn error of secretion may be the important factor in the not uncommon familial tendency to this cancer.

SMALL AND LARGE INTESTINES

The relative immunity of the duodenum and small intestine to cancer may again be due to destruction of all carcinogen-modified proteins by the natural

proteolytic enzymes there. This seems more likely than the alternative, quite plausible explanation that the stomach has borne the brunt of the mechanical, thermal, and chemical trauma and that the now homogenized mixture passes rapidly through the intestine and is never long in contact with any one area of mucosa. Conditions change abruptly in the large intestine. There are now in theory no proteolytic enzymes to nip the carcinogen-modified cells in early bud, and tumors are frequent. Contact of the mucosa with the more concentrated contents is prolonged; possibly, particularly at flexures, mechanical trauma is more possible, and maybe, potential carcinogens are excreted through its mucosa. Many carcinomas show first as benign polyps, which to me represent the first stage of neoplastic progression. Their more rapid growth is a reflection of diminished function resulting from the loss of identity protein, a loss which is still, however, not great enough to produce an immunologic distinction between tumor and normal tissue sufficient to allow invasion (failure of recognition and possibly aggression) to occur. If the exposure to a carcinogenic agent continues, further loss of identity antigens occurs, and a frank carcinoma ultimately appears.

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INHERITED CANCER SUSCEPTIBILITY

What of congenital diffuse polyposis with its frequent malignant change? Any explanation of this dominant inheritance should cover all cases of inherited tumor susceptibility which, while relatively rare in man, are commonplace in inbred laboratory mice. In immunologic terms we have to assume that the gene-controlled enzyme system for identity protein synthesis is unstable in the particular tissue and that at some stage failure occurs, or an abnormal (and therefore antigenic) protein is formed. This might explain dominant inherited tumors, but how far resistance or susceptibility to tumor formation may be related to more recessive types of inheritance is yet unknown. Obviously both individual animals and men vary widely in their response to known carcinogenic agents, and such variations must be of genetic origin, though, as with many other diseases, the genetic background is too complicated for present analysis. It is generally agreed that there is no evidence of inherited susceptibility to cancer as a whole, but inheritance of organ susceptibility certainly can occur. Obviously only when this becomes pronounced can it be detected in family histories. In terms of the immunologic theory, inherited factors might affect, for instance, the speed of adaptation of a carcinogen-modified cell to antibody attack or the power of the immune response to destroy precancerous cells and thus prevent cancer formation. On the latter point there is some evidence that high immunity of a nonspecific kind hinders chemical carcinogenesis and there is much yet to be discovered in this direction.

URINARY TRACT AND EMBRYONIC TUMORS

In the urinary tract we have complete evidence that many bladder tumors are due to chemical carcinogens concentrated in the urine, and we can suspect that others have a similar etiology. No doubt, promoting agents, particularly chronic infection, are important in this region. The tubular regeneration of chronic nephritis may also play a role as a promoting agent in hypernephroma

induction, but this is just another guess. Kidney tumors are a convenient point for considering tumors of early life, the embryoma of the kidney and the adjacent sympathetic neuroblastoma being by far the commonest of such tumors. I suggested[9] that they might be examples of displaced embryonic tissue whose identity protein(s) were, for some reason, still antigenic to its own organism and thus elicited an immune response which might culminate in malignant change. When we put forward this idea, the phrase "acquired tolerance" had not been coined. The idea restated would be that tissues which are liable to malignant change in early life are those which, owing to a delay in maturity, have retained some antigenicity to the body in general, i.e., have failed to develop complete acquired tolerance. If this were so, then the immune response set up, just as suggested in chemical carcinogenesis, could lead to a malignant adaptation. In favor of the hypothesis is the fact that tumors do tend to develop in tissues (e.g., sympathetic neuroblast) which reach maturity in postnatal life. Against it is the fact that embryonic tumors may occur in the fetus and therefore at a time when an immune mechanism possibly does not exist. This is a serious objection, but we are still inclined toward the idea that local failure in the development of acquired tolerance is linked with the genesis of this class of tumor. It is hardly likely that the tumors of early life are of quite a different order from all others.

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TERATOMAS

The teratomas though certainly of different origin from those just discussed, are perhaps easier explained on immunologic theory. However, the original focus of cells arises, whether by parthogenesis of normal or abnormally sited germ cells, or by segregation of very young pluripotential embryonic cells, the stimulus to differentiation comes in postnatal life, and complete acquired tolerance to these differentiated cells might not exist. So, in theory, the teratoma, originally an innocent malformation, would have a particular tendency for one or more of its component tissues to become malignant at some period after birth. Those like the ovarian teratomas which do not show this tendency, must be assumed to have existed in prenatal life in a diminutive but differentiated form.

LUNG CANCER

At the time when the talks on which this review is based were delivered, smoking and lung cancer had reached the newspaper headlines. It seemed to me then and still does that it was too easily assumed that the *cause* was a carcinogen in cigarette smoke. While ever there was a population exposed to the same hazard without the same result, this natural assumption was suspect. Particularly in human cancer it seems to me we have always to be aware of the old chronic irritation hypothesis with its obvious lesson that irritation alone is not sufficient. The irritant would now be called a promoting agent, which indeed has the more precise meaning of a special type of irritant, probably highly surface-active and capable both of progressively damaging the tissue and of stimulating a possibly specific hyperplastic response. As explained for excessive hormonal stimulation, promoting agents agents in general do not necessarily induce the shift to neoplasia by first altering the antigen make-up of the cell.

Rather do they promote those conditions which allow the antigen-deficient cell to emerge. This should be particularly prone to happen if, in the area affected, cells, already modified by previous exposures to a chemical carcinogen, exist. In industrial communities with the atmosphere laden with smoke, soot, gases, and fumes, legion in variety, how could the bronchial tree escape such an exposure? And yet this is usually insufficient (even over the life span), in the absence of cigarette smoking, to induce bronchial cancer. So we may assume that cigarette smoke, which undoubtably contains carcinogens, has a special promoting capacity and that the bronchial mucosa shows a high resistance to true carcinogens. In terms of the immunologic theory such variations in resistance to carcinogenesis in different organs, as with individuals and species, is to be expected. Long exposure to potent carcinogens like benzpyrene, abundantly present in soot, without resulting neoplasia argues that some defensive mechanism is at work. Cigarette smoke which is damaging to the bronchial tree, as heard in the chronic cough or as witnessed in the bronchial ulceration of the chain-smoker, appears to break down this resistance. That it does so by stimulating the outgrowth of initiated cells in an unfavorable environment with resulting further progression to frank neoplasia seems possible. That this stimulation is specific seems unlikely, and it seems more probable that it is a combined effect of several substances (not excluding carcinogens) in tobacco smoke which together possess the peculiar degree of irritative and destructive properties necessary in a promoting agent. Of course, the promoting agent is not invariably tobacco smoke, for it can be asbestos, hematite, and no doubt other inhaled materials.

We have dwelt on tobacco, however, not only because it is by far the most important single factor in lung cancer, but because it warns us against accepting too readily what may seem to be obvious conclusions about the mode of origin of human cancer. Assuming that tobacco smoking was a recently developed habit, one can imagine cancer workers saying it was only too obvious that vegetable matter pyrolyzed at such temperatures was bound to form strong carcinogens probably capable of inducing carcinomas of the skin, of the mouth, and of the whole respiratory tract. People would be so frightened that the habit would probably fail to spread and we should assume our prognostications were right. But in fact they would not be. As far as can be judged at present, a strong carcinogenic stimulus reaches, usually first at a very early age, the large bronchi as the result of industrial pollution. For some reason, possibly connected with the degree and duration of carcinogen contact with mucosa, the inhaled carcinogens would appear only to initiate and not to promote carcinogenesis. Any of a number of irritant substances may presumably promote these modified cells to frank cancer, but there is no certainty at all that carcinogens in cigarette smoke are the responsible promoting agents. On immunologic grounds any substance creating local conditions selectively favoring the outgrowth of cells deficient in some degree, however slight, in tissue-specific antigen, would promote neoplasia.

POSSIBLE VIRUS-INDUCED TUMORS

We have only glanced, as it were, with immunologically biased eyes at a few common human tumors. The often more widely disseminated tumors of

the lymphoid and hematopoietic systems are from this approach difficult to assess. Our work[15] on cancer anemia provides some evidence, as do more direct approaches, that such tumor cells lack specific antigens, and if this is so we can assume that the lack progressively increases from the lymphocyte of chronic leukemia to the blast cell of acute leukemia. The exciting causes are usually by no means clear, though exposure to ionizing radiations and various chemical carcinogens are important ways of increasing the incidence in susceptible strains of mice. Recently, a virus etiology has come strongly to the fore,[17] particularly in regard to mouse leukemias, though recent claims have been made that in human leukemia, transfer of a viruslike agent to mice is possible. The important point about these viruses is that infection is only transferred to the fetus via the egg or to the newborn infant by injection of infected material. It would seem to us that the story has many analogies with the Bittner mouse breast cancer virus and that it may be peculiar to certain strains of mice. Like this virus it may prove to have little bearing on the human disease. In case it should, however, it is as well to point out that the existence of tumor-inducing viruses is a strong point in favor of the immunologic nature of cancer. We have stressed[11,13] that it is the close structural relationship of the cytoplasmic viruses to the organ-specific antigens which allow them to be parasites. If they displace or replace such antigens by competition, then, whether or not they are themselves subsequently destroyed by the immune reaction, the net result is a cell actually or functionally lacking specific antigen and therefore automatically neoplastic. On the other hand, a so-called tumor virus need not necessarily initiate the neoplastic change. It can again, by virtue of its specific cytotropism, induce hyperplasia in the sensitive tissue and thus promote neoplasia in cells already in a precancerous state. The fact that in the case of leukemia, infection takes place only in the very young suggests that the virus acquires tolerance to the host and is thus able to infect certain blood cells at a later stage of life without attracting an immune response. Rather than being a primary cancer inducer it may well prove in this instance to be a promoting agent. In human leukemia neither a carcinogenic agent nor a virus may be essential for its development. For instance, chronic marrow damage by chemical agents (e.g., benzene) may result in atrophy followed by the emergence of leukemia. Here again one can visualize a profound hemopoietic stimulus to a badly damaged bone marrow causing an antigen-deficient white cell to emerge without any more specific process being involved.

How antigenic change, if this is in fact what occurs, is induced in the precursors of the malignant cells of Hodgkin's disease or the reticuloses generally, is far from clear. That cells which normally form antibody can be involved in the neoplastic change is, however, clear. This may explain the histologic picture of Hodgkin's disease which could be the result of an immune attack of the malignant cells on certain normal lymph node cells. This might even tend to protect the host by ultimately resulting in a fibrous wall which mechanically hinders the spread of tumor cells.

Progress in the more chronic of these conditions may be slow, but tends inexorably to greater malignancy which may be of relatively sudden onset.

Rapid death from acute leukemia may represent, in immunologic terms, a particularly vicious attack of the tumor on the host, the normal blood elements being rapidly destroyed so that the first line of defense is removed at one stroke. In other types of cancer the destruction possibly occurs more insidiously and may be evident only in the terminal phase of malignant cachexia.

CHORIONEPITHELIOMA

Although this does not pretend to be a complete survey of human tumors, that unique human growth the chorionepithelioma could hardly be omitted. Here is a tumor of one individual growing in another individual and therefore almost invariably immunologically different from its host. It is certainly for this reason that metastases occasionally spontaneously regress and probably why the diagnosis of clinical malignancy in the hydatidiform mole is so difficult. Invasion of malignant tissue often occurs but often, we would suggest, the host destroys it. So spontaneous cure of this malignant growth may be very much commoner than is thought. It would be interesting to know how close the genetic relationship between tumor and host must be before a tumor develops. A wide difference might always result in the death of the tumor cell soon after it appeared. It must be stressed that here we are dealing with an isoantigenic, predominantly nuclear, difference between host and tumor. The anemia which is sometimes a feature of this disease may be due to the tumor attacking host red cells with special force since it comes into very intimate contact with host red cells during its growth. Whether its etiology has an immunologic basis is an interesting speculation. If, as is possible, the gradual infarction and final separation of the placenta is due to an immune reaction by maternal tissues to the placenta, it would seem that retained placenta, if such an immunologic attack continued, might then be more liable to malignant change, the hydatid mole representing the first stage of antigenic loss. Such a theory would account for the apparently extraordinary speed with which this tumor can develop. The fact that the natural growth rate of the placenta and the intensity of the hypothetical immune reaction are both so high might, on rare occasions, allow of the development of a malignant growth in months (or weeks) rather than years.

TUMOR THERAPEUTICS AND IMMUNOLOGIC THEORY

Finally problems of therapy, particularly chemotherapy, have their bearing on the immunologic status of human cancer. Perhaps the most noteworthy feature is that many of the partially successful drugs have proved to be carcinogens. It is interesting that the first drug possibly showing some defined action on neoplastic tissue was arsenic, a carcinogen of low potential, and the first extensive nonsurgical method of treatment was by exposure to radium or x-ray, both potent carcinogenic agents. The main exceptions to this statement are drugs like substituted purines and folic acid derivatives which block essential syntheses in the cell and therefore tend to affect preferentially rapidly growing cells like tumor cells and normal marrow cytoblasts. The nitrogen mustards, triethylene melamine and urethane all have some temporary beneficial effect in the chronic, particularly lymphatic, leukemias. All are carcinogenic, and the

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first two, which also influence Hodgkin's disease as well, are potently so. It is noteworthy that it is the group of what were once debatable neoplastic states, the leukemias, Hodgkin's disease, and the reticuloses, which respond most favorably to these drugs. They include neoplasms which clinically give most evidence of the existence of some kind of systemic resistance to them. Their course is highly variable, they show remissions, often profound, and particularly in Hodgkin's disease, pyrexial episodes are frequent and often severe. It is interesting therefore that the only apparent responses to our own attempts to raise resistance to human cancer have been in Hodgkin's disease. Following the administration of noncarcinogenic coal tar fractions, a few advanced cases of Hodgkin's disease appeared to respond favorably with regression of lymphoid masses, body weight gains, appetite increases, and subjective improvements. Further trials with pure compounds from similar sources are still in progress. The present importance of this work is that it may help in understanding some of the factors which we have previously dealt with[13] concerned in the general resistance to the spread of spontaneous tumors.

Endocrine ablation therapy also has an important connection with the immunologic theory. The success of this treatment depends upon the tumor still being hormone-dependent. As we have often stressed[14] the hormone-mimetic and embryonic organizing actions of certain carcinogens together with a certain tissue-specificity suggest strongly that both hormones and carcinogens become biologically effective by combining with the same tissue-specific antigens. If the neoplastic tissue still retains some particular antigen which is the target for a hormone still essential for growth, then it will fail to grow in the absence of that stimulus. With further loss of this antigen hormonal independence occurs, and progressive growth is resumed with the greater vigor permitted by this loss.

This is only a sketchy account of the immunologic theory as applied to human cancer. I hardly need tell the reader that most of it is surmise and guesswork but some of the speculations may be useful. The theory as applied to experimental cancer is more satisfactory but this brief consideration of the human aspects may persuade some to look more closely into its possible implications in man.

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A CRITICAL ANALYSIS OF "TUMOR IMMUNITY"

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ALL laymen and many physicians think of the neoplastic process as a one-way street—untreated cancer progresses to an inevitable and predictable end. However, many close observers have been aware for a long time that, although most cancers are inexorable and progressive, a few are not. In fact, remissions are fairly common, cases of extraordinarily prolonged survival have been seen by most observers, and rare cases of regression are recorded. The last occur either "spontaneously," or without apparent adequate cause, in cases having histologic confirmation of the diagnosis. Stewart[1] and Boyd[2] have recently discussed such cases and cited examples. I have sometimes wondered if, through accidental circumstances, such cases may have bolstered the claims of quacks.

Although the rare regressions of proved cancer, in the absence of known adequate cause, furnish the most spectacular observations in this field, there are other reasons for a guarded optimism. Cases of unexpected remission and of unusually slow advance sometimes suggest relative but incomplete resistance on the part of the host, although many such cases may represent low or changing "virulence" in the tumor.[3] A stronger suggestion may be derived from the apparent behavior of tumor emboli.[4] Many observers believe that escape of emboli from a primary tumor is a relatively frequent occurrence. These emboli reach the blood or lymph stream and are transported to distant organs in fairly large numbers but only a part, probably a small part, succeed in establishing a clinical metastasis. Why? The real puzzle is not so much that metastases occur but rather why are there not more of them? A similar line of thought concerns the relatively small number of local recurrences in comparison to the large amount of "seeding" with cancer cells at operation (Smith[5]). The known difficulties confronting small groups of cells when, as transplants, they attempt to establish attachment and obtain nutrition do not seem sufficient to explain this. Nor does the argument hinge upon acceptance or rejection of Greene's ideas regarding "autonomy,"[6] although such a factor might be a modifying influence. Tumor emboli, or wound "seedings," are autografts. Autografts are expected to grow in a large percentage of cases, especially so if they are composed of neoplastic

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tissue. But most tumor emboli fail to grow. Could this be explained by some sort of intrinsic incompatibility, possibly mild, possibly of an immunologic sort, between tissues or between a tumor and its host? The existence of these unanswered questions is ample reason for a survey of this field and an attempt to find plausible working hypotheses among the scattered and confused facts.

The field of tumor immunity is poorly defined at present and comprises phenomena that are obscure and difficult to correlate. Definitions can rarely be given in precise terms, and one must usually be satisfied with descriptions in biologic terms. Even the use of the word "immunity" is subject to some debate. However, one must use words, and it is better to use old words, if they can be understood, than to coin new words prematurely. Much that will be said in this essay resembles immunology—it may actually be immunology in a broad sense. At any rate, it is convenient to use the terms of immunology in their broadest sense and, with the reader's indulgence, I shall so use them. Discussion of finer shades of meaning can be left to the future when more knowledge will make discrimination easier.

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The literature pertaining to this field is extensive, far too extensive to be covered in the space available. Fortunately, the reviews of Woglom,[7] Spencer,[8] Snell,[9,10] and Hauschka[11] leave nothing to be desired in the way of thoroughness. Consequently, the review that follows is not intended to be exhaustive. It is intended, in fact, less as a review than as the author's estimate of the present situation in tumor immunity. This material has been selected with the purpose of showing the broad outlines, the principal landmarks, and some of the implications of the field. The selection reflects the viewpoint of the author, although attempting to avoid bias. The regrettable omission of numerous worthy reports from the list of references cited is dictated by the need for covering a large field in a relatively small space.

If one speaks in broad terms, it may be said that three points of view have prevailed in experimental approaches to this field. For the sake of brevity these will be simplified (possibly oversimplified). The first presumes that the methods and concepts of classical bacterial immunity may be applied in a more or less direct manner to this field with a practical end result. The appeal of this approach is that it has a vast background of accepted knowledge and involves concepts that are relatively clear. Its main difficulty lies in the fact that for 50 years it has not worked except under special and limited conditions. The second viewpoint holds that certain tissues, e.g., the spleen, normally contain antitumor or antiblastic substances or potentialities and that extracts from them or related antisera might attack a tumor directly or augment host resistance. Spleen extract, duodenal extract, and antireticular cytotoxic serum are typical of this group. There is little to be said in favor of this group at present and, in the opinion of many observers, the premises on which the work was based are largely discredited. A related subgroup is represented by the bacterial toxins and enzymes that were said to arouse host resistance or attack the tumor. These, also, are not now in general favor. The third viewpoint is more complicated and has never been widely held, at least in its modern version, so far as I know. This viewpoint and the concepts and working hypotheses associated with it

draw heavily upon the published ideas of other investigators in the fields of tumor immunity,[7] genetics,[10] embryology,[12,13] immunology,[14] and the recently active field of tissue transplantation.[15] Only a sketchy description of this viewpoint will be attempted at this time because, being the author's viewpoint, it will be brought up again and, in addition, the many deficiencies in our knowledge make brief definition difficult.

The distinguishing feature of this viewpoint is the concept that cells carry some sort of information on or at the surface and that this information functions in various ways to influence the interactions of cells and their environment. By the term "information" I mean not only the chemical and stereochemical character of the molecules present but also the arrangement among the molecules; in other words, not only what is present but also how it is arranged. The ideas involved are similar to Landsteiner and Pauling's concepts in immunochemistry and also to Weiss's concept of "molecular ecology,"[16] but we attempt to extend these concepts to include the possibility that there may be a "cellular ecology" not necessarily limited to the molecular level. This can be done without detracting from the known facts regarding molecular influences in cells, both at the surface and inside. We entertain the possibility that such surface arrangements, or information, may be a factor in some of the obscure and elusive "individuality,"[17] "specificity,"[18,19] or "recognition"[20] systems that are encountered not only in tumor immunity but also in other areas of general biology. It is also thought possible that a fundamental disturbance in cancer may be a disarrangement of the cell surface and a consequent alteration in the cell's interaction with the host environment.

There are many scattered facts that seem to be in harmony with this viewpoint and, so far as I know, none that are contradictory. The reasons for thinking so will emerge from the survey which follows.

Half a century ago many workers seem to have been inspired by the idea that cancer could be detected or cured by means of a specific serologic reaction, such as had succeeded for other diseases. At that time, no genetically controlled materials were available, little was known about the etiology of tumors, and experimental results were highly and categorically varied. There was sufficient agreement, however, to make it evident that the search for serologic factors in tumor immunity would be difficult. The following facts indicate some of the difficulties.

It was observed that there was a universal natural barrier to transplantation of tumor from one species to another, but contrariwise, the tumors of one species would grow in vitro in the serum of almost any other species. In the heterogeneous mice then available, most mice could be made resistant against the implantation of most mouse tumors by a prior inoculation of either normal or neoplastic mouse tissue, but such immunity could not be transferred passively. An animal in which a transplanted tumor had regressed was solidly immune against reimplantation of the tumor, but attempts to demonstrate antibodies in the serum of such an animal failed. Animals that were demonstrably immune against one tumor might or might not be immune to another transplantable tumor, and they were never immune to autogenous or spontaneous tumors.

Against all these generally obtained results stood reports that stated the opposite.[55,56] Such assertion and contradiction characterized the work in tumor immunity for 30 years—and still does to some extent. In sum, there appeared to be a variety of situations in which immunity could be demonstrated easily but no antibody could be found. The suggestion that this is like tuberculin sensitivity can be met by recalling that specific tumor immunity (or immunity to skin) once established cannot be abrogated by irradiation, whereas tuberculin sensitivity can be so abrogated. In this respect immunity against tissues resembles bacterial immunity rather than tuberculin sensitivity.[21]

Information with regard to the antigen was also confused, and many additions to the literature served only to add to the confusion. Most workers agreed that against homologous (same species) tumors only homologous antigens were effective, but a few competent investigators reported the induction of resistance by heterologous (different species) tissues. Many reports indicated that resistance could be induced only by living tissue, but there were a number of reports to the contrary. Some even claimed that only dead or injured cells were effective. The latter idea led to unsuccessful experiments on human cancer. For example, despite Haaland's reports[22,23] that devitalization of tissues not only robbed them of their immunizing power but might also endow them with enhancing powers and that no immunity could be induced against an autograft of a spontaneous tumor, Kellock[24] attempted to immunize patients against their own tumors by means of irradiated autografts. The attempt was unsuccessful and was criticized by Wood.[25] A few investigators reported the induction of resistance by means of various tissue extracts and other noncellular materials. Against those stood reports that such treatment led to hypersusceptibility.[9]

It should be unnecessary to cite further examples to show that tumor immunity has become a highly controversial field. The work of early investigators produced some results that still stand as landmarks for later workers, but the principal product was a mass of contradictory literature—so much so that when Woglom reviewed the hundreds of contributions made prior to 1929, he said, "Of these communications a small number are of permanent value . . . and far too large a proportion are merely ridiculous . . . ." At about this time most workers who had been looking for an "anticancer" serum gave up the hunt and the field became unpopular.

However, at about the same time, new landmarks were emerging. Owing in large part to the efforts of Little and co-workers inbred animals came into general use. The powerful influence that genetic factors had exerted upon early experiments began to be appreciated and, based upon the work of Loeb, Tyzzer, Little, Strong, Bittner, and others, the genetic theory of transplantation was formulated.[26] This had the dual effect of burying the mistakes of the past and resurrecting some hope for the future; not the hope that an "anticancer" serum was in the offing, but the hope that something fundamental and useful might be learned about the manner in which a tumor and its host interact. Concurrently experimental embryologists, led by Spemann,[12] demonstrated that both the morphology and behavior of a cell may depend not alone upon what is in a cell but also upon its external associations.

New awareness of some of the basic influences at work in the field of tissue specificity suggested reinvestigation of some of the old problems as well as new problems to be attacked. The first requirement was better control of the experimental conditions. Control of the host was provided by the several strains of highly inbred animals that had become available. Rigorous control of the tumor could be had only by the use of transplanted tumors of known constitution, in spite of the known weaknesses of that procedure.[27] It is the employment of these principles that distinguishes modern work from old work in the field of tumor immunity, regardless of chronologic overlapping.

In the preceding paragraphs reference was made to the importance of the genetic principles involved in transplantation. Transplantation is important in this field for two reasons. In the first place, it has become increasingly apparent that the transplantation of tissue may provide an extremely delicate indicator of differences between tissues. In the second place, inbred animals and transplantable tumors derived from such animals are to the biologist what pure reagents are to the chemist. The only way that a cancer worker can perform two successive experiments with confidence that his reacting materials are the same is by employing such homozygous materials. I have said transplantable tumors and not spontaneous tumors because it is known that the spontaneous tumors of two similar animals, or even two simultaneous tumors of the same animal, may differ.[28-30] The use of spontaneous tumors involves variables that are partly uncontrollable and sometimes unsuspected. The use of transplantable tumors involves an oblique approach, but it seems for the present to be the safest approach to problems that must ultimately be solved with spontaneous tumors.

The genetic relationship of a tumor to its prospective host appears to be the most important among the factors that control the outcome of transplantation. Many other factors, mostly unknown, appear to exert an influence, but the genetic influence is primary, and experiments performed without attention to this point are apt to yield a meaningless result. As background for the material to be presented herein, it will suffice to recall the following principles.[26]

A tumor derived from a mouse of an inbred strain will grow when transplanted to any mouse of the same strain or to any F_1 hybrid mouse having that strain as one parent. It will not grow in mice of other strains or in unrelated F_1 hybrids except for certain exceptional tumors. On the other hand, a tumor originating in an F_1 hybrid mouse cannot be transferred to mice of either parent strain or to an unrelated hybrid, but it can be transplanted to all similar hybrids. In F_2 hybrid mice, procured by mating two F_1 hybrids, the genetic factors controlling transplantation segregate. A tumor from either of the inbred strain grandparents will grow in such F_2 hybrids in a percentage of cases that depends upon the number of factors involved—the larger the number of factors the smaller the percentage. Backcross mice are derived by mating an F_1 hybrid mouse to either of the parental inbred strains. Animals obtained by mating the hybrid to a mouse of the strain of origin of the tumor are called susceptible backcrosses; all such mice are susceptible to the tumor. Animals obtained by mating a hybrid to a mouse of the parental strain foreign to the tumor are called resistant backcrosses. Resistant backcross mice will accept the tumor in a percentage of

cases that varies from 50 per cent downward, depending upon the number of genetically controlled factors involved. No consideration is given here to such well-known but unusual tumors as Sa 37, Sa 180, Walker 256, etc. Their hereditary make-up is entirely unknown, and they appear to be partly specific and partly nonspecific, depending upon circumstances that cannot be analyzed at present. Different conditions prevail for those tumors known to be caused by a filterable agent. With such tumors, the outcome depends sometimes upon the transplantation of cells and sometimes upon inoculation of the agent. For that reason they are excluded here but will be mentioned again in another connection.

The genetic principles briefly reviewed seem to me to be the first chapter in a study of tumor immunity, despite the fact that, in the development of the field, they came relatively late. With these principles as guides, one may proceed to further explorations with some assurance of making a real advance in understanding what tumor immunity represents.

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It may be noted in passing that the principles just stated apply also to transplantation of normal tissues. Insofar as is known at present, the transplantation of normal tissues involves technical and quantitative differences, but the principles are the same as for tumors. However, they apply with either tissue only when the parent strains are homozygous (inbred) and not when the parents are heterozygous (noninbred, as in man). This has sometimes led to confusion in clinical attempts to apply these principles. This is not to say that genetic principles do not apply in man but rather to say that nothing is known of the genetics of man in this respect (identical twins excepted).

The profound, almost qualitative, influence that genetic interrelationships exert upon tumor immunity was shown by the following experiment.[31] Three strains of mice were employed as hosts for tumors, one a pen-bred albino strain (Rockefeller Institute) and two inbred strains (A and C57BLK). Three tumors, all mammary carcinomas, were transplanted, one of unknown genetic constitution (Bashford 63), and two that had originated in the inbred strains (15091-A and 755-BLK). Defibrinated blood from each of the three strains was employed as an immunizing agent. It can be seen in Fig. 1 that a wide spectrum of results was obtained. In general, when any two of the materials were held in a constant relationship, the results varied with the third. Furthermore, it appeared that the relationships were more important than the materials, because for any one of the materials, the outcome depended less upon the intrinsic qualities of that material than upon how that material was combined with the other two. The immunity involved appeared to be directed toward the "foreignness." The degree of "foreignness" appeared to be an expression of the genetic diversity of the materials.

Although it has already been indicated that the type of immunity under discussion has no known relationship to a cancer "serum," either diagnostic or curative, this aspect needs further emphasis. It is true that what we know about the reactions of an animal to the implantation of foreign tissues corresponds in broad outlines to bacterial immunity.[32] It is possible, however, that the similarity is overemphasized, because most positive observations have followed the introduction of extraneous tissue into a test animal. Such conditions are super-

ficially like bacterial invasion (in that both represent the introduction of foreign material), and there may be a tendency to emphasize gross similarities and obscure subtle differences. For that reason, as well as some others that follow, I prefer neither to accept nor to reject the analogies with bacterial immunity. In order to avoid cumbersome circumlocutions, however, it is convenient to use the terms of bacterial immunity. Their use has no etiologic, diagnostic, or therapeutic implication.

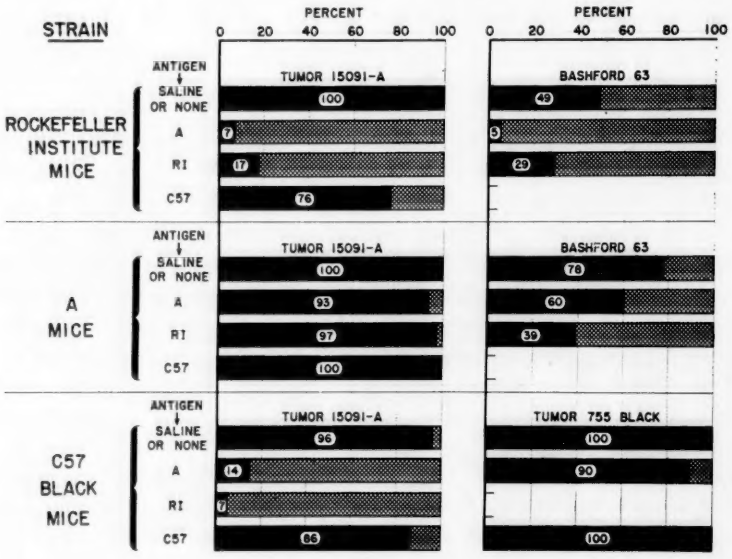

Fig. 1. Summary of data from some experiments in which resistance to transplantation of tumors was induced by the prior inoculation of homologous blood. The black areas represent the percentage of "takes" at 3 weeks. All hosts in each of the three rows were of the strain indicated at the left. All hosts in a line were immunized with the blood indicated at the left of that line. The tumor inoculated is shown at the top of each block. None of the differences in the blocks showing strain A tumors in strain A mice or strain C57BLK tumors in strain C57BLK mice is statistically significant. All other differences are significant except those between 7 and 17, upper left; 7 and 14, lower left; and 86 and 96, lower left. A total of 768 mice is represented on the chart.

Some other terms require definition. Many differences observed in this field are relative and the terms "immunity" and "resistance" will be used interchangeably. The term "natural" immunity will be used to indicate immunity observed in an animal that has had no known immunizing treatment. There is some doubt whether this type of immunity actually exists; it may represent a very rapid response to the stimulus of a test inoculation. This would be in keeping with the usual negative observations in tissue culture. The terms "induced" or "acquired" immunity are self-explanatory; they will be called "nonspecific" when the inducing agent is normal tissue. In using terms derived from "iso-," "auto-," "homo-," and "hetero-," common usage and the definitions of Snell[10] will be followed. Of those, the only ones that might cause any difficulty are

words like "isologous" and "isogenic"; as used here, they refer to the relationship between two individuals of the same inbred strain—a difference from classical serologic terms.

Many observers have reported that, although tumors can be transferred between individuals of the same species, there appears to be a natural immunity against transplantation between species of warm-blooded animals. With some qualification, that generalization still stands. The qualifications can be divided into several categories although, in the end, they may not be as diverse as they now appear. Murphy reported, in 1912, that tumors could be transferred successfully to the embryos of foreign species. This often-confirmed observation appears to depend upon the fact that, temporarily, the embryo is in some way immunologically deficient or incompetent. Later, the embryo or newborn animal develops the resistance that characterizes the adult; then the tumor must either be transferred to a new host, or it will be destroyed (unless "tolerance" has been induced[33]). The deficiency of the chick embryo can be altered by introducing a bit of spleen along with the graft. It should be noted that in this case the spleen of a normal homologous adult is effective.[34] In older animals, a special site may be used for transplantation. This is shown by Murphy's success in transplanting mouse tumors into the brains of rats. In this case it appeared that the *site* was immunologically deficient, because when the tumor encroached upon the ventricular wall, host cells invaded the tumor, and it was destroyed. The deficiency in this site could also be altered by introducing a bit of spleen along with the tumor. In this case the spleen of a normal adult was effective, but only when the spleen was *autologous;* homologous spleen was not effective, and neither were autologous testicle or blood. (This interesting report needs confirmation.)

At the expense of a slight digression, it is interesting to note that these very old observations strongly suggest some details of the most modern observations in protection against irradiation by bone marrow or spleen[35] and in "acquired tolerance" to homotransplantation.[33]

At this point it is appropriate to consider some recent observations of Weaver.[36] He investigated the growth of mouse tissues inside a small porous chamber, pervious to serum proteins but impervious to cells. The chamber, containing the mouse tissue, was placed in the abdominal cavity of a rat. In a normal rat the mouse tissue survived, but in a rat that had been immunized against mouse tissue the cells inside the chamber were destroyed. Survival of the cells in the nonimmune rat could be prevented by a bit of spleen in this case also. Bits of spleen from a mouse of a strain foreign to the tissue explant were included in some of the chambers. If the spleen were derived from a nonimmune mouse no effect was seen (within two weeks at least), but if the spleen came from an immune mouse (immunized with tissue isogenic to the explant) the transplant was destroyed.

It thus appears that the natural immunity between species can be circumvented by (a) using a very young host before it has developed effective defense mechanisms, or (b) transplanting into a site in which effective defense mechanisms do not seem to be elicited, or (c) imposing a physical barrier between the host and the transplant. In all 3 situations, the failure of the immune reaction could

be corrected by the inclusion of a bit of spleen (or also of bone marrow in the first case), but subtle differences are suggested by the different genetic relationships between the materials employed. For that reason it would not be wise to assume at this stage that the reactions are exactly alike.

It has been mentioned that certain transplantation sites may be immunologically exceptional. The brain, the testis, and the anterior chamber of the eye are examples. Special mention should be made of transplantation into the anterior chamber of the eye, a field to which Greene[6] has been a prolific contributor. (Greene's viewpoint differs from that of the writer, and his original papers should be consulted.) Most workers agree that heterotransplantation into the anterior chamber can be accomplished, but there is no agreement about the percentage of success to be expected or about the reasons for the failures. Lushbaugh[37] believes that the degree of success is low and that published reports tend to exaggerate the success. He believes further that the relative malignancy, or autonomy, of the tissue is not sufficient explanation for the success or failure. The author agrees with those opinions. Schilling[38] and the author (unpublished experiments) obtained a low percentage of takes in heterologous transplants. Eichwald[39] used the anterior chamber for homologous transplantation between several inbred strains of mice. He obtained a high percentage of initial "takes" but also a high rate of regression. He concluded that the natural barriers between strains were lowered but not abolished and that induced immunity was not bypassed. Medawar[40] transplanted skin homologously to the brain and anterior chamber of the eye in rabbits. He reported that in the brain the skin would respond to but could not elicit the immune state, and in the eye the transplant was destroyed if, and only if, it was penetrated by blood vessels. Finally, observations made by Merwin[41] should be mentioned; they deal with homologous transplantation into a different special site, but they are informative. She studied harderian glands after transplantation into a subcutaneous transparent chamber in a mouse of foreign strain. In this situation the graft usually became vascularized, the host was immunized, and the gland was destroyed after a short period, like the usual subcutaneous homologous graft. On the other hand, sometimes the graft did not become vascularized, hence no immunity was evoked, and the graft survived for long periods. However, if during such a period of survival a second graft became vascularized, then both it and the nonvascularized first graft were destroyed.

These findings indicate that the generalities of tumor immunity apply in these special sites but the reactions may be modified. It is possible that other special conditions exist, but one that appears to play a part is the failure of tissue to arouse the immune response when it is placed in special situations. If an immunity is already in existence, or if it is elicited by another stimulus, the graft in a special site is destroyed. This incomplete set of observations suggests that a transplant into one of the special sites reacts like a subcutaneous graft[41] that is not vascularized. Does this mean that, in a relative sense, the antibodies (possibly carried by white blood cells) are mobile in the absence of blood vessels and the antigen is not? At present it seems that this might be a partial explanation.

There are other methods for circumventing the natural resistance between species. The development of an effective immune mechanism usually requires some time, and workers have taken advantage of this by either transferring the tumor to a second host before the first host had sufficient time to destroy the tumor or by delaying the development of the immune reaction. The work of Putnoky[42] confirmed by Balogh[43] furnishes a good example of the former method. By transferring the tumor at short intervals Putnoky succeeded in maintaining a mouse tumor in rats for 7 years. There are three important points to be noted in regard to this sort of work. First, in the majority of cases the tumor will succumb to the defensive mechanisms of the host if it is left until these are fully developed. Cases have been reported in which the tumor persisted until the death of the host, but these can be evaluated differently according to one's viewpoint. The small size of the tumors and the early death of the animals raises considerable doubt. A second cause for doubt concerns the possibility that the tumor itself may have changed. Both Putnoky and Balogh reported that their tumors changed immunologically, and Murphy observed that a rat tumor appeared to change during residence in the chick embryo. Greene[44] considered the possibility that his success in transplantation into the anterior chamber of the eye might involve some degree of adaptation that eliminated the more radical constitutional differences between the transplant and the host. And finally, to the numerous reports of spontaneous enhancement of the ability of a tumor to grow after transplantation[45] may be added the author's demonstration that such a change can be produced at will.[46] The third cause for doubt concerns the question of simple survival. A decision on this question is most difficult. It should be recalled that Bullock[47] observed viable cells in rat tumors that had survived in mice for 54 days. In the universally unsuccessful transfer of homologous skin in man it is not uncommon for the graft to survive for a period of 5 or 6 weeks, and longer intervals have been reported. Gaillard[48] reported that homotransplants of embryonic skin remained intact for 6 months. Furthermore, during a period of survival a tumor may grow to large size; e.g., mouse tumors may reach 7.5 cm.^{2} in silhouette and then regress; that would correspond to about 5 or 6 Gm. Comparable rat tumors would weigh ten times as much. Thus it is known that a tissue that will ultimately succumb to its environment may survive for a long time and grow to a large size. The uncertainty is compounded by the fact that very little is known about how a tumor kills its host. When an animal dies *with* a tumor we can only infer that it died *because* of the tumor. Such considerations lead to the conclusion that the experiments cited did not demonstrate heterotransplantation except in a highly modified sense.

Returning to the question of circumventing immunity by delaying the development of the reaction or by interfering with its expression, there are numerous reports which show that this can be done by a variety of methods. Typical methods employ (a) irradiation of the host, (b) administration of cortisone, or (c) reticuloendothelial blockage. These have been extensively reviewed by Toolan.[49] No complete agreement with respect to the significance of this work can be reached at present but, in general, it appears that the principal influence is that of delay. One can adopt the viewpoint that the change is not so much

in the event as in its time of occurrence. During the period of delay the implanted tumor, the host, or both may be changing, and one is then confronted with the same sort of irresolvable complex as was outlined in the previous paragraph. However, there are some fairly well-established points that are worthy of note. Irradiation can break down natural immunity either between species or in homologous transplantation.[34] It can also prevent the development of induced immunity, either specific or nonspecific.[50] However, although Murphy reported some abrogation, most investigators agree that induced immunity cannot be abrogated by irradiation once it has developed.[49] In general, cortisone and ACTH produce effects that are similar to those of irradiation insofar as we are concerned here. In the case of reticuloendothelial blockage, a possible difference from irradiation has been reported. Ludford,[51] working with nonspecific induced immunity in mice, found that trypan blue would prevent the expression of a preexisting immunity, and Andervont,[52] working with specific immunity induced in mice by a tumor, found that trypan blue not only prevented the development of immunity but also abrogated the immunity after it had developed. However, the latter investigator noted that some of the animals were in such poor condition that dye injections had to be discontinued. It seems possible that the animals were so intoxicated that they simply could not muster such defenses as they possessed, as may occur in the presence of large tumors,[53] in which case the distinction mentioned would be of less importance.

The important point in all this is that agents that are known to have a deleterious influence upon immune reactions in general also influence tumor immunity in the same way. It seems unlikely that this is mere coincidence. However, the mechanisms involved remain obscure. Primarily the effect seems to be one of interfering with the development of immunity by action upon some part of the mechanism that initiates the chain of events leading to immunity.

Insofar as it can be demonstrated by present methods, the state of tumor immunity is limited in its effectiveness to certain situations. It is directed principally against the *establishment* of a foreign graft. It is not effective against an established tumor even when the established tumor and a second graft are from identical sources and placed in the same animal[54] (often confirmed by the author and others). It does not prevent the take of an autograft of a spontaneous tumor[55] but is effective against an autograft, i.e., within the same host, from an established homologous tumor,[52] provided that the latter is not isogenic to the host. Tumor immunity is usually not effective against transplantation within an inbred strain,[31] but in some cases subline differences may have an influence. Just where the line should be drawn in defining "foreignness" is not certain, but in present practice it usually lies in the region of differences between inbred strains and sometimes of differences between sublines within strains. Most important, the animal with induced immunity is not effectively immune against the development of spontaneous tumors. With regard to the last point it should suffice to mention the observations of MacDowell,[56] although other evidence is available. He worked with an inbred leukemic strain of mice and a transplantable leukemia, which had originated in the same strain but had been separated by many generations. Immunity against the transplantable leukemia could be induced (either specific-

ally or nonspecifically), but such immunity did not interfere with the development of spontaneous leukemia later in the life of the treated mice. This observation could be explained by assuming that the immunity induced early in the life of the mice had deteriorated by the time that the spontaneous disease arose, but it seems doubtful that such is the explanation in view of other evidence.

The phenomena of tumor immunity have been shown to bear considerable resemblance to bacterial immunity, but complete congruity is far from established. There may be a "natural" immunity, but this is uncertain, and in some ways it seems improbable. There is undoubtedly a state of "acquired" immunity, and it requires an incubation period for its full development. The immune reaction appears to be primarily an enhanced resistance to the establishment of a graft of foreign tissue and to be based upon the foreignness and not the neoplastic qualities of the tissue. The fact that both bacterial and tumor immunity can be forestalled by the same agents suggests a similarity in the initiating mechanisms. The fact that antibodies are demonstrated easily in bacterial immunity but have eluded detection in tumor immunity may mean that the effectors of the immunity are different in spite of a similarity in the mechanism of initiation. The few indications that humoral antibodies may be demonstrable in a heterologous system but not in a homologous system might suggest either a quantitative or a qualitative difference. Is a "unity of antibody" concept sufficient reason for discarding the latter possibility with respect to the homologous system? Or should the search for some special characteristic of tumor immunity be pursued?

As a concluding point in this attempt to characterize the immune state, brief reference should be made to "hypersusceptibility." Woglom was not convinced that such a state existed. However, in recent years, Casey, Snell, Kaliss, and others have adduced much evidence that there may be such a condition. They have treated animals with various nonliving tissue extracts and under varied conditions observed an increase in the number of takes of tumors, an increase in the growth rate, an increase in the number of metastases, a decrease in the survival time of the hosts, or varied combinations of these effects. There seems to be considerable specificity in the reaction. Snell has reviewed this work recently.[10]

The specificities encountered in a study of tumor immunity present some interesting features that may be helpful in characterizing this type of immunity. As in other features of tumor immunity, it must be kept in mind that specific effects are the product of a dual system, i.e., the primary influence may reside in the tumor, or it may be a mutual effect.

Many years ago it was known that, in general, an animal bearing a spontaneous tumor could be immunized against the implantation of a tumor from another animal but not against an autograft of its own tumor. This was not because the one tumor was intrinsically responsive ("avirulent") to immunity and the other not; a tumor that did not succumb to immunity in one host might do so upon transfer to another host. It was thus evident that demonstration of the immune reaction did not depend exclusively upon the properties of either the host or the tumor but upon the relation between the two. With the advent

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of inbred animals it became possible to demonstrate that this relationship depended primarily upon the genetic constitution of the materials and that this attribute accounted for much of the specificity observed.

Natural resistance to transplantation depends upon highly specific genetically controlled factors that appear to function as antigens. In order for a transplant to grow, it must not contain an antigen that is absent from the host. If a foreign antigen is present in the graft, the immune reaction is aroused, and the graft is destroyed. Apparently, the converse is not true; the absence of factors from the graft has no demonstrable effect. The early work of Tyzzer, Little, Bittner, Strong, and others established the fact that these factors are specific for strains,[26] they can be enumerated, their strain of origin can be traced when they occur in hybrids, and, in some cases, they are genetically linked with other characters. More recent work by Snell[57] has shown that specific alleles for histocompatability can be identified and that the specificity is a function of the allele so that two different strains may be the same or different for a given factor. These factors, and therefore the specificities, can change. There may be minor changes in the host that correspond to the development by spontaneous mutation of sublines within a strain. These have been discussed recently by Snell.[10] There may also be similar changes in a tumor which occur as spontaneous mutations[45] or as induced adaptations.[46] A brief discussion of the latter will serve to illustrate several of the points mentioned thus far.

A tumor, which originated spontaneously in an inbred animal, was transplanted into various types of hosts, and it was found that the tumor conformed to the usual genetic restrictions upon transplantation when the inocula were derived from stock tumors growing in the strain of origin. Under those conditions, 8 per cent of resistant backcross mice were killed by progressive growth of the tumor, indicating that probably four factors were concerned in that particular combination. However, if the tumor was first grown in an F_1 hybrid and then transferred to resistant backcross hosts, 30 per cent succumbed to the tumor (Fig. 2). Apparently, the specificity had changed so that only two factors were involved. This could be repeated at will, and further work showed that the change in the tumor tissue was permanent.[58] Continued study revealed that if two different genetic combinations were employed the changes observed not only differed in degree but also appeared to be specific for the strains used. There is not space here to go into all the complicated details involved in this work, but reference to the original publications will show that the specificity appears to extend to relatively small genetic differences, and some linkage with genes for color can be discerned. It seems probable that as knowledge increases a great many genetically controlled elements in tissue specificity will come to light and that they can be manipulated experimentally in some measure.

However, there are many situations that are difficult to explain in terms of a limited number of genetically controlled factors, although they may in the end be based upon some heritable property of the individual cells. For example, if one considers the so-called "nonspecific" tumors, which will grow in almost any mouse, theoretical difficulties arise. The stimulus for immunity is presumed to be the presence in the inoculum of an "antigen" which is absent from the host.

The fewer factors or foreign antigens present in the inoculum, the more widely transplantable the tumor and the less demanding the specificity. Extension of such reasoning (much of which is supported by facts) might lead one to conclude that the nonspecific tumors contained only antigens of a common, widely distributed type. It would follow that it should be difficult to immunize any mouse against them, and furthermore such tumors should be much alike in their immune reactions. However, the observed facts do not harmonize well with such a conclusion. A few of these observations follow.

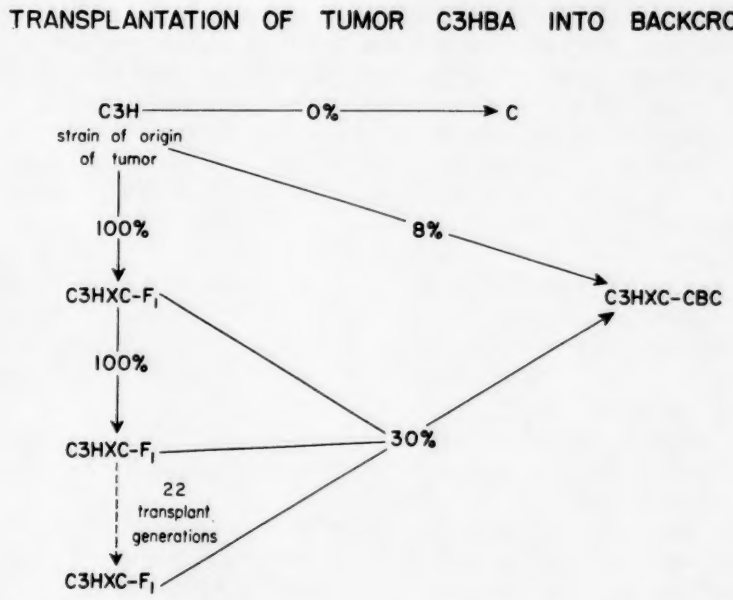

Fig. 2. Summary of the percentages of progressively growing tumors that were observed when the tumor was transplanted between animals of varied genetic constitution. The arrows represent the donor-recipient relationships. The percentage of hosts killed by the tumors is shown on the arrows. The differences are all highly significant; $P < 0.001$.

Almost any mouse may be immunized against these tumors, but various strains react in varied degrees, and for a given strain the reaction against different nonspecific tumors varies. This does not suggest a consistent "commonness."[59,60] Furthermore, a given tumor, such as Sa 180, may stimulate immunity against itself and others, such as Bashford 63 and Carcinoma A, but the last two will not stimulate immunity against the first. In experiments in progress, the author has made a similar comparison between Sa 37 and DBA 49, a sarcoma of known genetic constitution and limited transplantability. These observations suggest that Sa 180 (and Sa 37) is either more complicated immunologically or has acquired some special antigen, but these notions cannot be reconciled with the generally accepted concept that usually tumors lose immunologic factors as they become more transplantable. Another aspect of this situation is brought out in studies of resistance induced by normal tissues.[31,61] Although it appears that,

in general, blood used to induce resistance need have no known relation to either the tumor or the host in order to be effective, and although it is easy to induce such resistance against the widely transplantable tumor Bashford 63. I have not been able thus far to induce resistance against either Sa 180 or Sa 37 by means of a prior inoculation of blood. There seems to be no way out of this dilemma except to assume that either increased transplantability does not always represent loss of, or substitution for, effective antigens, or there is some unknown difference between the tissue antigens concerned in transplantation and some of those effective in stimulating immunity. Much work must be done before a decision can be reached on these points.

In summary, the phenomena of tumor immunity appear to be specific. The more easily discernible specificity appears to be based upon genetically controlled factors or antigens, and it has been estimated that the probable number of such factors is about 14.[10] Such antigens tend to function as strong antigens, in variable degree, and to exert a primary influence upon transplantation. However, it is difficult to explain all the variations by means of the limited number of factors that can be demonstrated in the usual experiment and, furthermore, the attributes of a tumor are not immutable in this respect. Whether the known heterogeneity of cell populations offers sufficient additional explanation is difficult to determine.[11] The author prefers to assume that although every cell is a more or less accurate replica of its progenitors there may be some variations in the phenomena under discussion that depend upon cell characteristics, or specificities, whose nature we have not yet guessed.

The search for antibodies has been difficult and has led to negative results for the most part. Some positive results have been reported, but they have not furnished a satisfactory solution to the problem.

Undoubtedly, a tumor contains many antigenic substances and, under suitable conditions, antibodies against them can be demonstrated. However, such a demonstration may leave several critical questions unanswered. Genetic factors must be taken into consideration in some observations. The classic example of cytotoxin aginst a tumor is found in the work of Lumsden,[62] who worked with heterogeneous animals. Phelps[63] demonstrated that in such animals the reaction depended upon the foreignness of the cells and not upon their neoplastic qualities. The observation of an antibody in any system which is heterogeneous may have nothing to do with a tumor as such. Another point which may cause difficulty is the presence in the tumor of some extrinsic agent. Probably all such agents are antigenic and neutralizing antibodies against some have been demonstrated, but Rous showed that resistance directed against a chicken tumor agent differed from that directed against the cells of the tumor.[64] Thus, if an extrinsic agent is present, antibodies may be observed that have little to do with the basic host-tumor relationship, although they may effectively prevent transmission of the tumor. These two difficulties may occur in a mixed form. For example, when dealing with chicken sarcoma one must consider not only an extrinsic agent, but also a heterogeneous population. Preparations consisting mainly of microsomes of Rous sarcoma and similar preparations of chick embryo pulp have been used to incite antibody formation in rabbits, and the antisera

so obtained were indistinguishable in complement fixation tests,[65] but it was possible to distinguish the antibodies directed against the tumor agent by appropriate absorption and tests in vivo.[66] These considerations suggest great caution in interpreting positive serologic reactions in this field.

Some information regarding the antibody and another possible complication can be deduced from the work of Murphy and of Weaver.[34,36] It will be recalled that Weaver saw indications of a humoral antibody when dealing with a heterologous system; none was seen, however, in the homologous system. The work of these investigators suggests a difference in the influence of bits of spleen that depended upon the genetic diversity between the materials employed. Another point concerns the inactivation of leukemic cells by either heterologous or homologous antiserum.[67,68] In the first instance, the cells and the host inoculated were homologous, but the antiserum was derived from a foreign species; in the second instance, all materials were homologous. In both cases, however, the inactivation was accomplished in vitro before inoculation of the cells. All such direct observations have involved an in vitro step, so far as I know, and this may be critical. It is uncertain how much difference these fine discriminations may make but it should be recalled that although the Rous sarcoma agent can be inactivated in vitro by antiserum, Rous[69] was unable to confer immunity by transferring nearly all the blood of an immune fowl to a fowl bearing the tumor. This is somewhat related to unpublished work of the author. I observed that rabbit antisera against a mouse tumor had a very high precipitin titer in vitro, but when 0.5 c.c. of such antisera was injected into the peritoneal cavity of mice it produced no observable resistance against a subsequent isogenic transplant of the tumor. This suggests that in the homologous system the antibodies differ either in kind or in degree to such an extent as to be difficult to detect, or there is some other difference in the mechanism.

The primary suggestion that tumor immunity may be mediated by a humoral factor comes from the long-established fact that the reaction is systemic. Prehn[70] and Malmgren[71] carried this postulate further by studying the immune reaction within transplanted tissues. By reciprocally transplanting tissues between immune and nonimmune animals and then making secondary tumor inoculations into the primary transplants they were able to show that the growth of the secondary tumors depended upon the systemic condition of the host and not upon the local conditions in the transplanted tissues. They recognized that if a sessile antibody had been transferred with the tissue it might have been obscured by the ingress of material from the host, but their findings show that the effectors of immunity are "mobile" and convert the primary graft to the same status as the host. Presumptive evidence of a similar sort has come from experiments on animals in parabiosis. The earlier experiments in this field either failed or, if they succeeded, could be explained by transmission of the antigen rather than the antibody. Bichel[72] suggested that some failures might be explained on a quantitative basis, because in his work the degree of communication between homogeneous pairs was much greater, as evidence by the transmission of cells, than that between heterogeneous pairs. Resistance was transferred under the former conditions but not under the latter. Falls' work[73] is subject to neither of

these criticisms. She induced immunity to a homologous leukemia in mice. Subsequently the immune animals were united with nonimmune animals of the same strain, after which the latter became immune. It is possible that transmission of cells is a critical factor in these experiments.

To sum up, it appears that the state of tumor immunity is systemic, but the nature of the antibody is not clear. In a heterologous system, a factor that can properly be called "humoral" seems to be involved, if one accepts the preliminary evidence available, but it is possible that this represents a simple cytotoxic response that has little relation to the central problems of tumor immunity. Whether this represents a qualitative or a quantitative difference from the homologous system cannot be determined at present. In the homologous system a "mobile" factor is involved, but the nature of the factor is not established. Experiments involving exposure of target cells in vitro seem to indicate a humoral factor but strictly in vivo experiments do not. A plausible explanation may be that in the homologous system, cells are required, either acting by themselves or in conjunction with some humoral factor.

The critical attributes of the antigens involved have also proved elusive, and this aspect of tumor immunity has suffered from the same confusion that pervades the other aspects. A generally held view has been that the ability to induce resistance against the implantation of a tumor is shared in varied degree by all or nearly all cells, whether normal or neoplastic. Most workers held that the critical property was inseparable from the living intact cells. A few thought that, although it was necessary to employ living cells, the active material was a metabolite of the cells, and some believed that the growth of the inoculated cells was necessary before immunity could be established. There was evidence that some antigens were more potent than others, and dosage effects could be demonstrated easily. All these points were subject to controversy.[9]

Tumors for which a filterable agent had been demonstrated presented special problems. As already indicated, the agent could be separated from the cells and in this state provided an effective antigen. However it was not certain whether this was a truly special case or whether there might be some relationship between this antigen and those of other tissues. There is not space for discussion of the large amount of work done in this special aspect, and the reader is referred to Hauschka's review[11] for a discussion of distinctive cell constituents in tumors.

Among the materials that may be used to induce immunity to tumors, the red cell is unique in several ways. It is easy to obtain in a relatively pure state and in any desired quantity. The dose is relatively easy to control. It contains no nucleus and appears to be simpler in structure than other cells. Much is known of its structure and composition. The employment of the red cell appears to exclude the question of growth of the inoculum and to reduce the question of metabolism to a minimum. Following in the footsteps of Bashford and his coworkers, we have studied the induction of tumor immunity by means of a prior inoculation of defibrinated blood and have succeeded in making a few advances.[61,74]

It has been found that homologous blood will induce resistance against homologous tumor transplantation, but heterologous blood will not do so even though hemolysins, etc., are produced. The blood cells are effective, but the

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serum is not. When the white cells and red cells were separated, it was found that both were effective in proportion to the numbers present. It became apparent that washed homologous red cells contained or carried an effective antigen. (Gorer[75] believes that the same genes control both histocompatibility and erythrocyte agglutinogens. This aspect will not be discussed here except to remark that these antigens might be associated without being identical.) The effect of laking upon the red cell antigen was studied by six different techniques; three of these resulted in nearly complete disintegration of the cell membranes, the other three left large quantities of ghosts. It could be presumed that all the methods were relatively gentle chemically. When the cells were subjected to freezing, or brief exposure to distilled water, or brief exposure to mild sonic vibrations, very few membranes or large pieces of membrane, remained. Such suspensions had lost their power to produce resistance. On the other hand, when the cells were laked by 0.3 per cent saline, or 0.02 M. citrate buffer, or rabbit antiserum, many ghosts remained, and such suspensions were as effective in inducing resistance as untreated cells. This made it clear that the induction of resistance required neither whole nor living cells. However, because disintegration of the cells ablated their power to induce resistance whereas partial disintegration did not, it was suggested that some degree of architectural integrity was required for the effective action of this antigen. It appeared that the required degree of organization of the antigen lay somewhere between that of a protein and that of a cell. It is possible that the nature of the antigen depends, not only upon its constituents, but also upon how they are arranged. This might be on a relatively large scale in comparison to other, better known antigens. These ideas harmonize with some work in which tumors were used as antigen. In previously cited work by others[22] and in briefly mentioned work by the author[76] it was found that disintegration of tumor cells robbed them of their power to induce immunity. This concept may also explain the fact that when implanted homologous tissue is separated from the host by a cell-impervious filter, no immunity is induced[77,78] since a cell fragment of the required size cannot pass such a filter. If this concept is correct, some past confusion may be explained. Almost any biologic inoculum contains one, often several, antigens of classical type. Among them a large, complicated, and labile antigen might be very elusive of detection even though important in the interaction of living tissues.

The foregoing analysis of the available facts in tumor immunity has not succeeded in bringing order out of chaos but it is hoped that the amount of confusion has been reduced. There is not space for detailed recapitulation of the various points brought out, but thoughtful consideration should show that much of the difficulty disappears if the viewpoint indicated at the beginning is adopted. Perhaps a brief restatement of the working hypotheses and concepts involved in that viewpoint is appropriate, but the formulation cannot be close-knit because of important gaps in present knowledge.

This viewpoint begins with acceptance of the observation that a few spontaneous tumors of man and of animals regress and that many tumor emboli fail to grow as autologous grafts. The reason for this is uncertain but it may represent some sort of resistance on the part of the host. The old doctrine of "horror

autotoxicus" can no longer be evoked as an argument against this, because it is now well established that autoimmunization occurs.[79] Historically this sort of "resistance" has been called "immunity." Indeed, it is instinctive and almost necessary to use the terms of immunology in discussing the field, but perhaps this is of itself misleading. The author[60] and others have found the term "immunogenetics" useful when confronted with the difficulties that Dempster[21] had in mind when he said, "The problems underlying homotransplantation of tissues have been traced to a no-man's-land where immunology and genetics meet." Some difficulty is avoided if the implications of the term "immunity" are broadened, as immunologists are themselves now doing. However, in this field, it seems necessary to stretch the terms to near the breaking point. As several have remarked, it makes sense to speak of immunity to bacterial invasion, but what is the biologic significance of immunity to implantation of tissues? The latter seems in many aspects to be a laboratory artificiality (except for some naturally occurring phenomena that cannot be discussed here for lack of space). However, in my opinion, the artificiality resides in the methods of study, imposed by present limitations of knowledge, and not in the phenomena. Some, but probably not all, of the many aspects of these phenomena may be related to broader aspects of biology and naturally occurring phenomena. There is satisfying evidence that living cells interact with each other and with their environment in specific ways and that some of the interactions modify cellular structure and physiology, tissue morphology, and organization. The best known examples of this are found in the field of embryology. Perhaps if these are kept in mind along with the broadening viewpoints of immunologists some of the seeming artificiality and confusion of transplantation immunity may, in time, disappear.

Cells express their potentialities in ways strongly modified by their morphologic position and associations. Experimental embryology is replete with observations indicating that whether a cell differentiates, how it differentiates, and to what extent depends upon environmental factors. There is evidence to indicate that some, if not all, of these intercellular reactions are mediated through some sort of "information" carried by the cells. Experiments already cited suggest that some of this "information" is on the cell surface, and some of it may depend upon relatively large and complicated arrangements of the constituent molecules. If that is so, it would not reflect upon indications that lesser fragments of whatever is "arranged" may also be active or upon the probability that because of their simpler nature they might be first to be demonstrated and studied. If such "information" ("antigen") is involved in tumor immunity, as it appears to be, it is not characteristic of the tumor as such because it is shared by normal cells. However, it is conceivable that one of the attributes of malignant cells is a disarrangement of such "information" so that the cells no longer react normally to their environment.

On a tentative and speculative basis, one may look upon the sparse facts of tumor immunity as fragmentary representations of broad biologic principles, broader than is usually implied by the term "immunity." It is possible that in the past we have been dealing only with very special cases of general phenomena which should be thought of in relation to development, differentiation, and or-

ganismal controls, as well as in relation to immunity. It was this sort of speculation that led to the search for evidence of some sort of "specificity" in gastric digestion.[19] Experimental results encouraged retention of the working hypothesis that "recognition" systems may be widespread in biologic phenomena, as others have also suggested. How such systems operate is a mystery for the most part at present, but there are many facts to support the belief that they may be an important influence in cellular behavior. Although the term is loose and rather uninformative, cellular behavior poses the central problems in cancer and in other biologic phenomena. All factors influencing cellular behavior are important and will remain so until our knowledge of cancer is complete.

To some readers, this viewpoint may seem somewhat mystical, to others teleologic. It really is not. On the contrary, it attempts to relate some of the artificialities of transplantation immunity to the realities of general biology and thereby remove some of the obscurity. The real test of the value of any working hypothesis is whether it leads to experiments that yield interpretable results and suggest further investigation. The viewpoint expressed here has led to such experiments in the past and may do so in the future. It also has the philosophical virtue that it keeps open another possible approach to the problems of learning what a cancer is fundamentally and what might be done about it practically.

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RECENT STUDIES ON PATHOPHYSIOLOGIC MECHANISMS IN HUMAN NEOPLASTIC DISEASE

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THE effects of a neoplasm on the host may range from disturbances so subtle that only fortuitous observation leads to their recognition to disturbances which are dramatic and disconcertingly obvious. Distortion of normal structures and of anatomic relationships, epithelial ulceration with bleeding and infection, and organ destruction with functional impairment are among the direct mechanisms which produce easily understandable compromise of normal tissue physiology by the advancing tumor. Less well known are the indirect effects of the growing neoplasm on the dynamic equilibrium of the host, effects which may be as disastrous as those due to direct tissue invasion.

In the succeeding sections an introduction will be given to recent work which has added to the description and partial understanding of the pathologic physiology of neoplastic disease in man.

ENDOCRINE TUMORS

It has long been recognized that tumors of endocrine glands may be functional, leading to the signs and symptoms of intoxication from excess hormone production. All of these will not be discussed here since the majority are well described in monographs on endocrinology.[1,2]

Argentaffinoma.—In the last 5 years a new syndrome produced by a tumor of endocrine tissue has been recognized, although the neoplasm itself was described more than 75 years ago. In 1867, Langhans[3] and, in 1888, Lubarsch[4] reported on rare tumors of the gastrointestinal tract which arose from chromargentaffin cells in the crypts of Lieberkühn. Despite the fact that it is now well known that these tumors can and frequently do metastasize widely,[5] it was formerly thought that they were benign neoplasms because of their morphology and, therefore, were called carcinoids.[6,7] Unfortunately, the latter name is more popular than the more precise designation, argentaffinoma. In 1953, Isler and Hedinger[8] noted the association of chronic endocarditis with pulmonic valve stenosis and tricuspid insufficiency in 3 patients with ileal argentaffinomas metastatic to the liver. Thorson and associates,[9] in 1954, described in detail the syndrome associated with carcinoids which can include periodic flushing of the skin, a purple

red cyanosis, intestinal hypermotility with diarrhea, bronchoconstriction, and asthmalike symptoms, as well as congestive heart failure due to the abnormalities of the right side of the heart recorded by Isler and Hedinger. In addition to the careful clinical description of 3 cases personally observed, these authors collected cases from the medical literature which had similar signs and symptoms. On the basis of the isolation by Lembeck[10] of 5-hydroxytryptamine (serotonin) from a human argentaffinoma, Thorson and his co-workers[9] suggested that the carcinoid syndrome was due to the production of this hormone by the tumor cells.

The accuracy of this suggestion was quickly confirmed by the demonstration of abnormal quantities of 5-hydroxytryptamine in the plasma and urine of patients with this tumor as well as by the recovery of the degradation product of serotonin, 5-hydroxyindoleacetic acid in the urine.[11-15] In large part, the carcinoid syndrome can be ascribed to the pharmacologic actions of 5-hydroxytryptamine,[16-19] but the pathogenesis of the cardiac lesions still remain obscure. It has been suggested that these are due to local irritation and that the left side of the heart is not affected because the hormone is metabolized to 5-hydroxyindoleacetic acid by monoamine oxidase in the lungs; this attractive hypothesis was not supported by the findings of Sjoerdsma, Weissbach, and Udenfriend[20] who failed to find a pulmonary arteriovenous difference of plasma serotonin. Since it is known that the 5-hydroxytryptamine is synthesized by the argentaffin cells from tryptophane,[21] a part of the clinical syndrome may be due to a tryptophane deficiency.

Primary Aldosteronism.—An even more recently described functional endocrine tumor is an adrenal adenoma which may produce the syndrome of primary aldosteronism. This disease was first recognized and described by Conn.[22] Before reporting the brilliant observations of Conn it may be well to trace briefly the scientific history which made the description of primary aldosteronism possible. In 1950, Deming and Luetscher[23] found a substance in the urine of patients with nephrosis and congestive heart failure which had strong sodium-retaining activity as determined by bioassay. Chromatography established that this material was not desoxycorticosterone. In 1952, Tait, Simpson, and Grundy found a material biologically and chemically similar in the "amorphous fractions" of adrenal cortical extracts.[24] A collaborative investigation by Simpson and Tait working at the Courtauld Institute, Middlesex Hospital, University of London; Wettstein and Neher, Ciba, Ltd., Basle; and von Euw and Reichstein, University of Basle led to the isolation of the 18-aldehyde of corticosterone, or aldosterone, in 1953.[25] Independently, Mattox, Mason, and Albert[26] also crystallized the hormone.

In 1954, Conn[27,28] related the known pharmacodynamic effects of aldosterone to a syndrome in a patient and made a preoperative diagnosis of an adrenal cortical tumor. This was confirmed at operation, and with unilateral adrenalectomy a reversal of the physiologic abnormalities was achieved. For 7 years, the patient had been having intermittent attacks of intense muscular weakness, occasionally so severe as to produce "paralysis" of the lower extremities. In addition to the muscular weakness, but not temporally related, the patient had

spasm of the muscles which by description were typical of tetany and could readily be corrected by intravenous calcium gluconate. Hypertension, polyuria, and polydipsia were also prominent in the clinical picture. The laboratory studies revealed hypokalemia, hypernatremia, elevation of the serum CO_2 and pH. Conn proposed that the syndrome could be explained by an excessive secretion of aldosterone which led to abnormal sodium retention and increased potassium excretion. The muscular weakness followed from loss of intracellular potassium in the muscle. The hypokalemic alkalosis resulted from a disturbance of the intracellular cation pattern with replacement of hydrogen ion as well as potassium by sodium and the elaboration of an acid urine despite hypernatremia. The mechanism of the tetany could be related to the alkalosis rather than hypocalcemia. The hypertension remained unexplained.

In 1956, Conn reported on 12 cases of primary aldosteronism which he or others had diagnosed.[22] Nine of the 12 had been cured by surgical removal of an adrenal cortical adenoma. It is probable that many cases diagnosed in the past as potassium-losing nephritis[29] were also due to an adrenal tumor. Additional case reports of aldosteronism due to adrenal cortical tumors are being reported in the literature.[30-33]

Thymoma.—Several syndromes have been related to tumors of the thymus.[34] Since the normal function of this gland has not been determined it is not entirely certain that the syndromes and the thymic tumor are more than fortuitously associated. In some instances, however, removal of the neoplasm is followed by remission of the systemic disease, and for this reason it would appear justifiable to present the observed relationships, at least tentatively.

In 1877, Wilks first described the clinical entity which later became known as myasthenia gravis.[35] In 1901, Weigert[36] noted the association of a thymic tumor with myasthenia, and Castleman and Norris, in 1949, summarized their experience at the Massachusetts General Hospital and that reported in the world literature with the finding of 97 thymomas in 330 cases of myasthenia gravis.[37] Although it is tempting to ascribe to the thymus the capacity to elaborate a curare-like substance which produces myasthenia, no such material has ever been found. Further, despite earlier hopes that thymectomy might be of value in the management of myasthenia,[38] the results have been discouragingly irregular so that operation cannot be recommended for the treatment of this muscle disease although it may be important to remove a potentially malignant anterior mediastinal tumor.[39,40]

Another interesting but still tenuous association between thymoma and systemic disease is that of erythrocyte agenesis. Opsahl[41] reported a tumor of the thymus in a 56-year-old man with bone marrow aplasia and myasthenia gravis. In 1945, Humphreys and Southworth[42] instilled new interest into the problem when they reported complete cure of severe anemia following surgical removal of a benign thymic tumor. Since 1928, at least 14 cases have been reported in the literature in which agenesis of erythrocytes and tumor of the thymus have been the constant characteristics of the syndrome.[43-49] In 2 patients, agammaglobulinemia was also noted,[50,51] but in the rest the necessary studies were not conducted to determine the frequency of this protein deficiency. About one-

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half of the patients with erythrocyte aplasia have had surgical thymectomies, and 50 per cent of these have had benefit.

It can only be concluded at this time that the syndromes of myasthenia gravis, red cell agenesis, and agammaglobulinemia in association with tumors of the thymus provocatively suggest that these, too, are examples of distant effects of neoplastic cells on host function.

The syndromes described in the preceding paragraphs serve to illustrate the capacity of certain tumors to exert their influence on body physiology through elaboration of biologically active substances, a mechanism which may be operative more frequently than has been generally considered.

PHYSIOLOGIC CHANGES IN MULTIPLE MYELOMA

There are other tumors which are known to produce substances capable of modifying profoundly the physiology of the host. Prominent among these is multiple myeloma. This neoplasm produces the well-known symptoms and signs of bone pain, pathologic fractures, hypercalcemia, and pancytopenia through growth within the medullary cavity and progressive skeletal destruction.[52] In addition, however, the abnormal plasma cells elaborate several types of proteins which account for many of the pathophysiologic manifestations of this disease.[53] Ninety-eight per cent of patients show a distinctive protein abnormality in the serum or the urine or both. The electrophoretically homogeneous serum protein migrates within the mobilities of gamma to alpha-2 globulins. The urinary protein has a smaller molecular size and may or may not have the heat-coagulation properties described by Bence Jones.

The constitutional manifestations of multiple myeloma which are related to the abnormal proteins can be categorized as secondary to intravascular phenomena or to tissue deposition. Within the circulation, coating of the erythrocytes by the myeloma protein produces rouleau formation and rapid sedimentation. This may, on occasion, lead to impairment of central nervous system function through the formation of multiple small thrombi secondary to red cell sludging. A more common expression of the protein effect on the erythrocytes is shortening of their life span through sequestration and destruction in the spleen. This, probably, is a more important mechanism of the anemia of multiple myeloma than replacement of erythropoietic cells in the marrow by tumor.[54,55] On occasion, the abnormal serum protein in multiple myeloma is a cryoglobulin, and signs and symptoms result from the precipitation of the protein in peripheral vessels.[56] A final recognized intravascular disturbance produced by the dysproteinemia is bleeding in the absence of thrombocytopenia. The mechanism for this phenomenon is the adsorption of proteins necessary for blood coagulation on the myeloma protein.[57] On this basis, hypoprothrombinemia and interference with the conversion of fibrinogen to fibrin has been demonstrated.

Significant functional disturbances, and ultimately uremia, secondary to deposition of Bence Jones protein in the renal tubules is a common occurrence in multiple myeloma.[58] In addition to this well-recognized consequence of extravascular localization of an abnormal protein, there is another systemic manifestation of myeloma which at least in part is due to such a mechanism. This is

the varied syndrome of para-amyloidosis. The glycoprotein may occur as perineural or intraneural deposits producing the symptoms and signs of polyneuropathy; deposition in connective tissue areas as, for example, about the wrist joint may produce pressure on the median nerve as it passes under the ligaments and lead to the carpal tunnel syndrome; the heart, gastrointestinal tract, and kidneys may be infiltrated with para-amyloid leading to intractable cardiac failure, gastrointestinal bleeding, or uremia. At the present time it cannot be stated with certainty that the glycoprotein of para-amyloid is entirely a product of the myeloma cell or whether components of the connective tissue involved also contribute to its composition.

There is a final known protein disturbance in multiple myeloma. Characteristically in this disease there is a depression or absence of normal gamma globulin.[53] This has been correlated with frequent pyogenic infections in patients with myeloma.[59] But it is to be emphasized that in many instances impaired antibacterial defenses are not evident despite apparent absence of normal gamma globulins. The mechanism for the suppression of the normal globulin synthesis is unknown.

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HYPERTROPHIC OSTEOARTHROPATHY; POLYCYTHEMIA

There are a number of other pathophysiologic manifestations in which products of the tumor are circumstantially implicated. Clubbing of the fingers and toes with or without the subperiosteal new bone formation of hypertrophic osteoarthropathy is a frequent sign of primary pulmonary cancer. This impressive and often painful soft tissue and bone syndrome is reversible following surgical removal of the tumor or effective radiotherapy. Although such a sequence of events suggests the elaboration by the tumor of a substance acting peripherally, there are many facts which make this explanation dubious. Clubbing is a nonspecific manifestation occurring as it does in pulmonary suppurative disease, cyanotic congenital heart disease, subacute bacterial endocarditis, biliary cirrhosis, myxedema, ulcerative colitis, and other diseases. It has been postulated that increased peripheral blood flow or tissue hypoxia play a part in the pathogenesis of the condition, but these are frequently not apparent.

Another phenomenon associated with certain malignant and benign tumors is polycythemia. This has been observed to occur in patients with renal carcinomas[60-65] and also in association with fibromyomata uteri,[66,67] benign kidney tumors,[68] and neoplasms of the brain.[69] Following surgical removal of these tumors the polycythemia gradually disappears. In the case of the renal tumors it has been suggested that the abnormal growth may lead to the release, or increased production, of erythropoietin. No direct evidence for this mechanism has been presented, and alternative possibilities such as arteriovenous fistulae within the tumor vascular bed have not been excluded. That this type of secondary polycythemia is not specific for tumors is shown by the demonstration of reversible polycythemia associated with marked unilateral hydronephrosis.[70]

HYPERCALCEMIA

One of the occasional complications of neoplastic disease is hypercalcemia with attendant disturbances in critical renal and central nervous system functions.

In the vast majority of instances of hypercalcemia the disturbance in mineral metabolism is due to skeletal metastases with delivery of the cation to the circulation at a greater rate than it can be cleared by the kidney. The symptoms and signs of hypercalcemia are often so nonspecific that an opportunity to modify the natural history of the disease, at least temporarily, is overlooked. Nausea, vomiting, and constipation appear early in hypercalcemia. These manifestations, associated with polyuria due to the renal effects of the calcium ion, rapidly lead to dehydration. The toxic action of calcium on kidney function is not well understood, but only rarely is nephrocalcinosis seen in the hypercalcemia of neoplastic disease. More important is the decreased renal blood flow[71] and an effect on the cells of the tubules which eventually results in extensive epithelial necrosis.[72] The dehydration and nephrotoxicity of hypercalcemia are quickly reflected by the chemical findings of azotemia and, shortly thereafter, the symptoms of uremia. There is another pattern of hypercalcemia which is less well known than the sequence just described. In this, impairment of cerebral function predominates with disorientation, psychotic episodes, somnolence, and coma. This syndrome can occur without uremia and may reflect the action of the calcium ion on synaptic transmission.[73]

The development of hypercalcemia in neoplastic disease metastatic to the skeleton can be correlated with the biology of the tumor. Thus carcinomas of the breast, lung, and multiple myeloma are most frequently involved in the production of this complication. Although carcinoma of the thyroid metastasizes to the skeleton with regularity, hypercalcemia rarely occurs because the tumor grows so slowly that renal excretion of the calcium liberated from bone prevents accumulation in the serum.

There is an additional mechanism of hypercalcemia in neoplastic disease which, although infrequently encountered, is provocative. This is the development of elevated serum calcium concentrations without evident skeletal involvement. In the past several years, this has been described from several clinics.[74,75] In the largest series observed[76] there were 3 patients with symptomatic hypercalcemia and serum concentrations greater than 16 mg. per cent who had a prompt return to normal levels following surgical removal of the tumor. In 2 cases the tumor was a hypernephroma, and in 1 case it was a carcinoma of the ovary. That this phenomenon is not peculiar to renal and ovarian carcinomas is indicated by the fact that in more than 12 additional patients with disseminated tumors including lung, lymphoma, endometrium, kidney, and ovary, hypercalcemia without bony metastases was observed. These latter patients showed no gross or microscopic evidence of skeletal metastases at autopsy despite sections of multiple samples of bone. In the patients with hypercalcemia without evident skeletal involvement the serum inorganic phosphate was within the normal range until uremia appeared, the erythrocyte sedimentation rate was consistently markedly elevated, and the alkaline phosphatase was only occasionally above normal. Hyperplasia of the parathyroid glands or skeletal changes of osteitis fibrosa cystica were not found. The precise mechanism for this type of hypercalcemia is not known, but circumstantially it is suggested that a tumor product is acting on the skeleton as a humoral agent.

ANEMIA

Anemia, among the most regularly recognized manifestations of neoplastic disease, may clearly be due to blood loss or nutritional deficiency. It is likely that in the majority of patients, however, these mechanisms are not operative. With the availability of appropriate isotopes, it has been possible to examine previously popular concepts of toxic depression of erythropoiesis by tumor products and also the possible contribution of an increased rate of red cell destruction. In ferrokinetic studies it was found that 69 per cent of patients with neoplastic disease had normal or accelerated rates of red cell production as measured by the rate of appearance of erythrocytes with isotopically labelled hemoglobin.[77] From this and ancillary observations on the plasma iron disappearance curve, it was concluded that suppression of erythropoiesis did not play a major part in the anemia of neoplastic disease in the majority of patients. It has, however, been observed that deficient red cell production does occasionally occur in cancer patients even though extensive medullary metastases are not demonstrable.[78]

Using the technique of differential agglutination described by Ashby[79] it has been shown that normal erythrocytes transfused to patients with neoplastic disease have a reduction in their anticipated life span in about 60 per cent of the patients studied.[80,81] This shortened life span is greatest and most frequent in patients with leukemia and lymphomas[82] but also occurs in patients with disseminated epithelial tumors.[54] Labelling the patient's own erythrocytes with Cr^{51}, and studying their life span when reinfused, demonstrated the same findings as using red cells from normal donors. The finding of an increased rate of erythrocyte destruction has been made in patients with disseminated but not in those with localized disease. It has also been found that as there is advancement of the disease, the erythrocyte life span is progressively shortened, thereby explaining the increasing difficulty of maintaining hemoglobin with transfusions in the later stages of neoplastic disease.[77] It is also noteworthy that following the production of a remission by chemotherapy[83] or by surgical removal of a tumor[84] the hemolytic rate may be decreased.

Studies of the life span of red blood corpuscles from patients with neoplastic disease transfused into normal recipients indicated a normal life span in two-thirds of the cases.[85] This suggests that an inherent corpuscular defect is probably not an important factor in the shortening of the red cell life span and reemphasizes the probable importance of humoral or tissue sequestration mechanisms which are operative in the tumor-bearing patient. In patients with an increased rate of erythrocyte destruction demonstrated by following the concentration of their own Cr^{51}-tagged cells in the peripheral circulation, Ultmann[55] has found significant localization of the isotope in the spleen. Since sequestration of the labelled erythrocytes in tumor masses or liver did not occur, the evidence suggests that mechanisms similar to those operative in other acquired hemolytic anemias may be important.

In summary, it is the current consensus that there is an increased rate of erythrocyte production and an increased rate of erythrocyte destruction in the majority of patients with neoplastic disease. The development of anemia is the consequence of a "functional inadequacy" of erythropoiesis because red cell destruction exceeds the rate of production.

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CENTRAL NERVOUS SYSTEM AND SKELETAL MUSCLE

The direct involvement of the central nervous system by intracranial metastases, of spinal cord compression from extradural masses, or of peripheral nerve roots by infiltrating tumor is unfortunately commonplace. Major disturbances of central and peripheral nervous system function from hypoxia, hypercapnia, hypercalcemia, uremia, cholemia, or hypokalemia can occur as a part of the complex natural history of a neoplastic disease. More subtle are degenerative changes in the nervous system which may occur without any associated generalized metabolic disorder. Brain, Daniel, and Greenfield[86] first described a series of patients who had clinical signs of cerebellar and posterior column disease manifested by dysarthria, gross ataxia of the upper and lower extremities, diplopia, and cramp-like pains in the legs. At autopsy, degeneration of areas within the cerebellum and the posterior columns of the spinal cord was found. In addition, these patients had either bronchogenic carcinoma or carcinoma of the ovary without central nervous system metastases. Denny-Brown[87] has similarly recorded degeneration of anterior horn cells in several patients with bronchogenic carcinoma without evidence of vascular compromise of the spinal cord by metastases or poliomyelitis.

The numbers of patients involved in these bizarre syndromes is not recognizably large, but the observation of Thompson[88] of a patient with bronchogenic carcinoma and truncal ataxia whose central nervous system signs were reversed following radiotherapy to the tumor lends support to the concept that the degenerative changes noted are a systemic effect of the abnormal growth rather than fortuitous intercurrent disease. Waravdekar[89,90] has conducted a series of interesting experiments which may have relevance to these human observations. When an appropriate tumor is transplanted into rats there is a very rapid inhibition of diphosphopyridine nucleotide (DPN) synthesis in liver and in blood hemolysates. This is reversible following surgical extirpation of the tumor or its successful therapy with a drug. If this effect on DPN synthesis extends to the central nervous system, and occurs in man as well as animals, it might be anticipated that the glycolytic pathway, which is DPN dependent, would be modified. Since the central nervous system is exquisitely susceptible to change in carbohydrate metabolism, functional and anatomical defects might occur. This is entirely speculative at the present time.

Brain[91] has also reported on weakness and fatigability of skeletal musculature with atrophic paresis most marked in the limb girdles and proximal muscles of the extremities in patients with neoplastic disease. Eaton and Lambert[92] have described a similar myopathy in 6 patients with motor end plate disturbances resembling myasthenia gravis and appropriate symptoms even including increased susceptibility to curare. Five of the 6 had malignant intrathoracic tumors, and none of them responded to Prostigmin. In our clinic Thompson has observed this type of myopathy in 5 patients with malignant neoplastic disease; it is indistinguishable from muscular dystrophy except for the age of the individuals affected. In 1 patient with bronchogenic carcinoma and a completely disabling myopathy, radiotherapy to the tumor was associated with

gradual return of muscle function. Five months later evidence of tumor recurrence and symptoms of myopathy were temporally related.

At the present time it is difficult to state without equivocation whether or not the remote effects of tumors, and particularly bronchogenic carcinoma, on the nervous system and skeletal muscle are real or only apparent.

CARBOHYDRATE METABOLISM

Disturbances in the disposition of carbohydrate have been reported intermittently in cancer patients for many years.[93-95] Results of such studies have been difficult to interpret because of the uncontrolled influence of such variables as fever, starvation, liver, and renal disease. Marks and Bishop[96] have recently reinvestigated this problem by studying the intravenous glucose tolerance curve of patients with a variety of tumors. Rigid criteria of selection were used which would effectively exclude those factors known to modify glucose metabolism. The neoplastic diseases included chronic leukemia and lymphoma in remission, as well as localized carcinoma of the cervix and breast. In more than 75 control and experimental subjects studied, the rate of glucose removal from the blood was found to be significantly slower in the tumor patients than in the controls. The mean fractional rates of glucose disappearance were 4 per cent for the normal subjects and 2 per cent for the patients with malignant neoplastic disease. The mechanisms involved in this disturbance are not certain; measurement of the serum phosphate and potassium concentrations during the glucose tolerance study showed no consistent differences between the experimental subjects and the controls. The decrease in blood sugar following a standardized dose of insulin showed a slower rate of glucose disappearance in the patients with tumors than in the controls. This finding, together with earlier studies by Young, Abels, and Homburger[97] which showed impaired liver glycogenesis in patients with gastric cancer, favors a disturbance of peripheral utilization rather than excess contribution of glucose to the blood by the liver.

MISCELLANEOUS

This completes a brief summary of recent studies relevant to a description of the pathologic physiology of the tumor-bearing human host. It is well recognized that the review has omitted several topics. The oft-quoted association of migratory thrombophlebitis with carcinoma first reported by Trousseau, in 1861,[98,99] and the coexistence of collagen diseases with certain neoplastic diseases in a greater frequency than might be expected to occur by chance alone,[100,101] have not been discussed because little can be said about possible mechanisms involved. The valuable conceptual and experimental observations by Mider and his associates[102,103] on energy and nitrogen metabolism in tumor-bearing rats which defined the tumor as a "nitrogen trap" have had repeated confirmation in human metabolic balance studies.[104-107] Recently Mider and his associates[108,109] have reported experiments on the mechanism of anorexia in the tumor-bearing rat. The results confirm earlier preliminary results of Mider[110] and Haven and Bloor.[111,112] From the evidence presented, it is suggested that the anorexia asso-

ciated with a growing tumor may represent a depletion syndrome since the feeding of lyophilized tumor tissue to a tumor-bearing rat with a reduced food intake rapidly increases his food consumption. These intriguing experiments may have relevance to an important symptom in human neoplastic disease.

SUMMARY

Progress is being made in the description of disturbances in the physiology of the patient with cancer. For certain problems such as the anemia of neoplastic disease and many of the phenomena of multiple myeloma, knowledge of the mechanisms involved has increased rapidly. In other areas, the issues have been defined, and work on precise mechanisms is going on. Studies of the type described in this communication hold promise for offering rational measures to support the host against the effects of the tumor, for defining criteria which may be used objectively as indicators to measure therapeutic regimens and for broadening the understanding of the biology and biochemistry of the tumor cell.

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THE NATIONAL CANCER CHEMOTHERAPY PROGRAM

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INTRODUCTION

THE establishment by the United States Government of a national program of chemotherapy research has aroused much debate in many quarters. Even now, two years after the program operations began, the program is questioned from time to time by segments of the academic world, the pharmaceutical industry, and the medical profession. As time passes, however, these skeptical questions are becoming less frequent, and the debate is giving way to a growing acceptance of such a program. Basic research sponsored under the program is flourishing in academic laboratories, the pharmaceutical industry is launching major drug development projects, and the medical profession is responding with extensive clinical trials of potential anticancer drugs.

Today the national program is without precedent in size, scope, and complexity, and there are indications that it may be setting a pattern for a number of similar drug development programs in other chronic diseases. Indeed, at the request of Congress, there are already the beginnings of such programs in the fields of mental disease and hypertension. In view of these facts it appears to be a propitious time to take stock of the program and what it has accomplished.

HISTORY AND ORGANIZATION

Until as recently as 15 years ago, chemotherapy of cancer was still the target of scientific skepticism. A serious lack of funds, facilities, and trained technicians to carry out adequate screening programs fostered disinterest in university laboratories. Private industry, feeling the pressure of commercial competition, was reluctant to risk money on what looked like a dark horse scientific gamble. Many cancer experts felt that chemotherapy offered little hope of success.

Others, convinced that chemical treatment would be the ultimate answer to disseminated cancer, initiated programs of cancer chemotherapy research. Among the first were Shear, Rhoads, Huggins, Farber, Lettré, and Yoshida.

From these and other research projects have come several dozen drugs with anticancer properties. For example, full, though temporary, remissions in chronic leukemia, Hodgkin's disease, and other lymphomas can be achieved using such alkylating agents as nitrogen mustard. Antimetabolites, such as methotrexate

and 6-mercaptopurine, produce spectacular temporary remissions in about half of the cases of acute leukemia. Methotrexate has completely suppressed metastatic choriocarcinoma in a few patients for periods ranging from 3 months to 2 years. Male hormone produces remission in about 1 of every 5 cases of metastasized breast cancer. Castration plus stilbesterol produces remission in 80 per cent of patients with metastatic cancer of the prostate. Cortisone is useful for temporary therapy of acute leukemia. Antibiotics, such as actinomycin D, though extremely toxic, are beginning to be used in the clinic.

These advances led the Congress, in 1955, to approve a national cancer chemotherapy program which combined the efforts of the National Cancer Institute with those of the Food and Drug Administration, the Veterans Administration, the Atomic Energy Commission, the American Cancer Society, and the Damon Runyon Memorial Fund for Cancer Research. The Cancer Chemotherapy National Service Center was formed to coordinate and administer the program.

The sponsors established the Cancer Chemotherapy National Committee to facilitate communication among the sponsoring agencies and to make recommendations concerning the cooperative research program. Because of industry's special experience and skill in drug development programs, its participation in the cooperative program was greatly desired. Therefore, the National Committee organized the Industry Subcommittee to advise on the promotion of industrial participation. Five scientific panels—Chemistry, Screening, Pharmacology-Biochemistry, Clinical Studies, and Endocrinology—were formed to give the program direction.

The result was a highly integrated cooperative scientific operation which insured complementary, rather than competing, research programs.

THE VOLUNTARY COOPERATIVE APPROACH

There is probably no field of medical research which requires more varied skills than drug development. One must have available biologists, chemists, pharmacologists, biochemists, endocrinologists, engineers, microbiologists, statisticians, nurses, pharmacists, and clinicians. Furthermore, their efforts must often be coordinated. Such extensive teamwork is rare in academic research where the emphasis is on independent, free-roving, individual investigation.

Ordinarily one finds such organized teams only in the research and development laboratories of major pharmaceutical manufacturers. Even there the team usually lacks extensive permanently organized clinical trial teams. And industry had shown little interest in the field of cancer chemotherapy until recently.

Because of the need for integrated research, one of the first problems in the national program was that of deciding how to obtain the necessary teamwork. There were those who favored an engineered, directed approach like that of the wartime malaria program. Under such a program an expert committee is given control of funds and is assigned the job of directing the research of those investigators and laboratories willing to participate. This approach was rejected almost immediately as being both psychologically and scientifically unsound. It was considered psychologically unsound because neither the academic nor the

industrial researcher willingly accepts bureaucratic direction by the Federal Government except in time of war or similar crisis. It was thought to be scientifically unsound because of the evident need for a high degree of originality, which is a quality seldom displayed by committees.

For these reasons the entire program is based on a network of hundreds of independent investigators. They are not required to work together and do so only on a voluntary basis when the team approach seems the best way to reach a desired goal. The Cancer Chemotherapy National Service Center and its panels strive constantly to identify areas which seem to call for teamwork. Having identified such areas, the Service Center takes the initiative in finding appropriate investigators and provides means whereby these men may meet to consider the problems and plan the necessary cooperative program.

In many instances, the independent investigator requires large-scale, expensive technical services which he is unable to provide or to obtain in his own institution. The synthetic chemist needs screening services, the screener needs large numbers of high quality mice, and the clinician needs a rather large quantity of each new drug properly formulated for parenteral use. The Service Center devotes much of its time, energy, and funds to provide such services.

On the other hand, certain types of research require teamwork from the very beginning. Examples include large-scale, empirical drug screening programs, definitive clinical trials, and statistical studies of the natural history of human cancer. How the Service Center handles such problems on a voluntary cooperative basis will be described in the next section.

Empirical Drug Screening.—The largest integrated activity in the national program is that of routine, empirical screening of chemicals and natural products for possible anticancer properties. To accelerate the empirical search for effective agents, the Cancer Chemotherapy National Service Center has established probably the largest drug screening program in the history of medicine.

Under this program are offered routine anticancer screening facilities free of charge to competent chemists and organizations throughout the world. The materials are accepted, and results are sent to suppliers on a confidential basis. The supplier retains full rights in the materials and is encouraged to follow up any "leads" which may develop in whatever manner he sees fit.

The materials are screened in one of 8 new screening laboratories under contract to the Service Center. The flow of materials to these contractors is controlled by the Service Center, and the methods used are those recommended by the Screening Panel. Tumor screens used are the solid subcutaneous forms of sarcoma 180 and adenocarcinoma 755, and the ascitic form of leukemia L1210.

Synthetic chemicals, plant extracts, and antibiotic culture filtrates are being screened for anticancer activity at a rate of 40,000 per year. There are two major sources of such materials: (1) the off-the-shelf selection of chemicals from educational institutions, research organizations, and manufacturing concerns; and (2) active programs of synthesis based on existing leads arising from compounds that have shown activity. Such programs of active synthesis are in operation in several pharmaceutical houses, or have been supported in other institutions by grants-in-aid or by contracts.

Many of the synthetic chemicals, and almost all of the culture filtrates, come from pharmaceutical companies. To accelerate screening, the Service Center has arranged contracts with some companies to supply and test their own materials.

The availability of the eight routine screening laboratories to provide such services makes it possible for the Service Center to enlist the active participation of more than 100 chemical and pharmaceutical companies as well as hundreds of academic and government chemists.

The results of this voluntary cooperative approach have been most encouraging. Many highly competent chemists are now working for the first time in the cancer field and many new leads are turning up.

A major activity related to routine screening is that of routine hormone assays provided on a similar service basis. Two contracts provide for service assays. Approximately 1,500 assays were made in the past year.

Another important item is the batch production of new chemicals in sufficient quantity to permit extended biologic and clinical study. To meet this need, the Cancer Chemotherapy National Service Center (CCNSC) has contracted for the operation of several small-scale pilot plants in which kilogram quantities of known chemicals are prepared.

Definitive Clinical Trials.—The outstanding example of the value of the voluntary cooperative approach is found in the clinical area.

The high degree of variability in the clinical course of different kinds of cancer and even in different individuals suffering from morphologically identical types of cancer is too well known to require elaboration. Because of this biologic variation it is difficult to evaluate therapeutic regimens. This is especially true in testing new drugs in which the differences in response may be rather slight. The situation is further complicated by the difficulty in predicting which type of cancer may respond to which drug.

If one is to move with speed and desires a reasonably high level of confidence in the results, it is necessary to study rather large numbers of patients representing a variety of tumors under carefully controlled conditions. To do so requires the combined efforts of a number of clinical investigators. The Service Center has approached this problem on the voluntary cooperative basis. Small groups of 6 to 12 investigators are called together to explore the organization of a cooperative group. Usually they are selected on the basis of medical specialty, geographic location, and demonstrated interest in certain forms of cancer. After explaining the problem and the reason for calling the group together, the Service Center leaves the rest to the group. In this manner approximately 20 groups representing some 165 hospital services have been formed and are studying more than 40 different chemicals in about 1,800 patients.

Natural History of Human Cancer.—Another example of the voluntary cooperative approach is the setting up of retrospective and prospective statistical studies of the natural history of human cancer—untreated as well as treated by various modern methods.

The ultimate test of any therapeutic regimen must be the effectiveness of its end results. Chemotherapy of cancer must ever be viewed in the light of its

relative ability to reduce morbidity and mortality as compared to other forms of therapy.

To obtain information of this general nature is the objective of the end results study now going on under CCNSC auspices. The study is a voluntary cooperative program involving approximately 20 hospital and 3 state tumor registries. Under a plan developed by the cooperating investigators, each register supplies duplicate punched cards for each cancer case in each registry to a central unit where the results are analyzed. From this study will emerge a picture of the response of various types of cancer to various forms of therapy. The data will be useful in planning future studies, may serve at times in lieu of concurrent controls, and will demonstrate the effect of changes in therapy as they are extended into general use.

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FINANCIAL SUPPORT

Financial support comes largely from Congressional appropriations to the National Cancer Institute, and the increase each year in the amount of money allotted to the program clearly reflects the Government's growing confidence in chemotherapy research.

When the program began in 1955, Congress earmarked only $5,000,000. In 1957, however, the amount rose to $20,000,000. This year the chemotherapy appropriation was $25,000,000.

In addition to government appropriations, the program is also financed by grants from the other sponsoring agencies.

The National Cancer Institute funds are divided about equally between research grants and research contracts. In general, research grants support individual, independent basic research projects in academic institutions, while contracts support applied and developmental work in commercial and industrial laboratories.

Research Grants.—Usually the initiative for a research grant project comes from the investigator. He applies for a grant, competes on a merit basis with other applicants, and if successful is granted funds to pursue his research as he sees fit.

The staff and technical panels of CCNSC do not participate in the review of proposals or the administration of grants. This arrangement was established to assure preservation of the academic freedom of the grantees.

Fundamental research is supported in any area which seems likely to have a bearing on cancer chemotherapy. Examples are: biochemical studies of normal versus cancer cells; studies of the influence of hormones; studies of the biology of animal tumors, of cancer cells in tissue culture, and of human tumors in heterologous hosts; studies of drug mechanisms and drug resistance; and studies of growth regulation. There are approximately 200 grants for such studies at an annual cost of $4,000,000.

Applied research in drug development is also supported through grants to academic institutions. There are 80 projects on drug synthesis, 50 on drug screen-

ing, and 75 on clinical evaluation. A few institutions like the Sloan-Kettering Institute and the Children's Cancer Research Foundation finance their integrated programs of cancer chemotherapy largely through research grants.

Financing independent research by grants has proved effective over the years and goes a long way toward meeting the needs of the academic laboratory. It has serious defects, however, as the sole financial device in a well-balanced, aggressive research program of drug development. In general, scientists apply for support of biochemical studies of drug mechanisms and of small-scale, exploratory clinical studies, leaving untouched the other essential aspects of drug development.

In most fields of experimental therapeutics this imbalance is compensated by extensive industry-supported programs of drug synthesis, drug screening, preclinical pharmacology, and definitive clinical trial. Unfortunately, in the cancer field this has not been the case. It has been necessary to provide these activities by special means, such as contracts.

Research Contracts.—This activity contains much of the "programmed" research of CCNSC and the national program. In general, contracts support the applied and developmental research initiated by CCNSC upon advice of its panels. (The main exception to this is the large-scale, cooperative clinical trial which we finance through grants.) Proposals are invited by the Service Center, are reviewed by one of the panels and subsequently by a Contract Review Board, and are finally converted into active contracts by negotiation between the National Institutes of Health and the prospective contractor. There is often a substantial degree of governmental supervision of work carried out under contract (Table I).

TABLE I. CONTRACTS APPROVED AS OF MARCH 1, 1958

CATEGORY	NUMBER OF CONTRACTS	AMOUNT
Synthesis of new agents	16	$1,927,641
Screening and screening methods	44	7,727,321
Hormone assay	4	649,395
Pharmacology	3	325,801
Clinical trial	6	638,300
Tumor registry	13	177,081
Total*	86	$11,445,539

*Does not include approximately $300,000 expended for the purchase of chemicals.

Although most of the contracts in effect or under negotiation are with nonindustrial organizations, pharmaceutical companies are showing increasing interest in active participation in the national program. A number of firms now have contracts, and others are negotiating with the Service Center for contracts which provide for such activities as "in-plant" screening, research on methodology, and preparation of chemicals.

The industrial program is being approached on the same voluntary cooperative basis. Each company is encouraged to develop a research program which

reflects the special competence and scientific interests of the company. Insofar as possible, each company finances its own program with minimal support from the National Cancer Institute. The importance of competition between companies is recognized, and companies are not expected to cooperate with their competitors. Every opportunity is afforded, however, for cooperation between the company and other nonindustrial laboratories.

Special attention has been given to the problem of preserving for each company its commercial rights to products developed at company expense. In return, the companies have made available many chemicals along with extensive biologic data at an early date and often before the chemical is protected by patents.

In recognition of the special patent problems inherent in research and development contracts with pharmaceutical firms, the Department of Health, Education, and Welfare has adopted a special patent policy for this area. In general, the policy provides that so long as Government funds support only drug testing, each company retains full rights in its own compounds. If Government also supports drug synthesis or natural product isolation in a pharmaceutical company then the policy requires agreement that a royalty-free license be granted the Government to use any invention made under the contract. It provides further that the company may retain ownership of patents resulting from drug synthesis contracts without Government interference so long as the company and its licensees supply the market with a product of high quality in adequate amount and at a reasonable price. The precise manner in which Government would remedy the situation should a company fail to comply with this requirement is still under discussion, but an early resolution of this aspect now seems probable.

Meanwhile, the companies are financing drug synthesis entirely with company funds but are accepting contracts for drug testing and related research.

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SUMMARY

Despite a somewhat stormy beginning, a large-scale national cancer chemotherapy research program has been launched in a relatively short time. Industry, government, and academic institutions are working together with increasing effectiveness and enthusiasm.

The underlying philosophy has been that necessary teamwork could be achieved on a voluntary basis. The importance of scientific freedom in academic institutions has been emphasized as essential for assuring originality and diversification. Industry has been encouraged to participate in a manner calculated to preserve the traditions of competitive free enterprise. Government has attempted to provide funds, services, stimulation, and an organizational framework in which cooperative research could develop. Whether the program will succeed from a scientific standpoint remains to be seen. It seems reasonable to conclude, however, that the underlying philosophy is sound and useful in organizing peace-time target research programs in the health field.

REFLECTIONS FROM CANCER CHEMOTHERAPY SCREENING STUDIES

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"ALL that glisters is not gold." This old proverb most aptly describes the true character of certain substances uncritically advanced as cancer chemotherapeutic agents. It equally well describes untried or inadequately proved test procedures that possess an aura of attractiveness not matched by their utility. Disappointments from such samples of "fool's gold" will naturally give rise to questions concerning experimental cancer chemotherapy studies, particularly in the minds of those who may be practically interested in the useful products of such studies without being familiar with the techniques in their development.

What is experimental cancer chemotherapy screening? How is it conducted? Of what value is it? An attempt will be made to answer some of these and related questions without detailed discussions of results or techniques which have been reviewed elsewhere.[1-3] The discussion will consider the interdependent aspects of chemotherapy studies, namely the test objects, the test procedures, the test materials, and the evaluation of results. Clinical considerations of cancer chemotherapy will be covered by others in this symposium[4] and recently have been reviewed more extensively[5] and thus will be referred to in the present report only to add significance to the discussion of experimental studies.

The objective of experimental cancer chemotherapy is to find substances with a differential toxicity, an adequately greater toxicity for tumor cells than for normal cells. It is the purpose of cancer chemotherapy screening to achieve the objective as rapidly, as reliably, and hence, as economically, as possible. From Webster we could fashion a definition of screening as the separation of the coarser from the finer parts. A screening procedure, whether it separates sand from gravel, heavy particles from those less dense, or the more promising antitumor candidates from the great bulk of inactive compounds, will depend for its performance upon the nature of the screen and the materials fed into it. Thus, an evaluation of the results of any screening procedure must consider what objective can be achieved by its experimental design and how adequate qualitatively and quantitatively has been the raw material fed into the system. We have used the term screening in experimental cancer chemotherapy to apply to

procedures that select from a large number of tested materials a small group worthy of further study in experimental cancer chemotherapy. The definition is pertinent regardless of the test object, the method of test, and whether the candidate materials are synthetic compounds or materials of natural origin, such as products from living plants or from cultures of microorganisms.

Cancer chemotherapy studies go beyond screening. Included are many investigations that some have called secondary screening, follow-up studies, etc. Regardless of how they may be designated, studies comprising a logical follow-up of a confirmed screening result include: titration of the active material to provide a therapeutic index, tests in various tumors including spontaneous tumors as well as transplanted tumors of various ages to determine the breadth and intensity of the antitumor activity, tests in resistant tumors and other studies to provide possible information on the mode of action, and pharmacologic studies prior to clinical trial. Such studies have been intimately tied in with some of the screening programs as a desirable part of the screening endeavor.

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Cancer chemotherapy goes beyond the investigation of single substances and their practical trials in man. It embraces the use of biologic agents (such as viruses), immune mechanisms, and of combination therapy. Combination therapy, although still relatively young, includes combinations of chemicals with other chemicals in which the added drug may protect the first from excretion or destruction or promote its absorption. The increase of effect from these actions probably will be accompanied by increased toxicity. A better combination prospect—of increased effectiveness without a parallel increase in toxicity—might be the result of interference in metabolic pathways, either sequential or alternative, of such pertinence to the cancer cells that there would be a true synergistic result. Combinations of chemicals with radiation may be more effective through additions of actions, through making the radiation itself more effective, or through protecting tissues from the effect of radiation. Chemicals as adjuvants of surgery are coming in for increasing study, and special methods may be required for selecting the adjuvants. Mouse leukemias and tumors that metastasize readily should be particularly useful for trial of agents sought to be effective against free cancer cells in the body, whether they be normal metastases or liberated by surgery. It is also not too much to hope that in the future immune mechanisms may be possible additives to the effects of chemicals in man; however, it is well recognized that the cancer cell as a variant from the normal represents less of an exploitably different cell whether considered biochemically or immunologically than do other invading parasitic cells.

It is well recognized that if selective susceptibility, the concept of the basis for successful chemotherapy (and in particular cancer chemotherapy), were followed to its ultimate conclusion, all screening of compounds would be done in man. As this has not been feasible, other biologic systems, including other cancer-bearing hosts, have been used to search for a biologic activity in a monocellular system or for a selective toxicity in the more rigorous practical challenge of a multiple cellular system in the hope that the materials so chosen would prove useful against at least one form of cancer in man. We are faced, therefore, with the question of what system is a good substitute in the selection of candidate

materials for human study. Are rapid, easily conducted assays with microorganisms, or with cancer cells utilized in microbiologic techniques of sufficient reliability to dictate their wider use? The degree to which cancer chemotherapy screening is conducted may suggest the desirability of such additional prescreens. The large national program in cancer chemotherapy is expected to screen at least 50,000 materials in the next year.[7] This bears a relation to previous screening efforts much as placer gold mining does to a number of individuals panning for gold. With several million dollars involved in screening with standard procedures in animals, it is clear that a simple, inexpensive technique, such as a microbiologic assay, if used to eliminate reliably even 10 to 25 per cent of the materials to be screened, could save substantial sums of money, large numbers of mice, and considerable human effort.

With respect to tests against cancer in experimental animals many questions are raised. With the variations in responses of various experimental tumors to the antitumor agents now available, which tumors are the most useful? What may be the relative merits of spontaneous, induced, and transplanted tumors? Is any one tumor of sufficient predictive value, or are many likely to be required to minimize to an acceptable degree the chances of missing a useful agent? Is any of the apparent lack of usefulness of any test tumor or test procedure merely a reflection of the failure to test appropriate materials? The differences in responses among experimental animal tumors have been emphasized frequently, and the principle has been recognized in the national program through adoption of a triple tumor screening program. In addition to increasing the prospects of detecting antitumor activity in screening with a group of tumors, a tumor spectrum can provide other useful information. It permits a better definition of the possible breadth of activity of an agent: it may provide a more sensitive test tumor than the tumor initially used to detect activity: it may provide a better comparison of closely related compounds.

It had been considered that ascites tumors with their greater sensitivity might be able to replace multiple tumor screens. However, while to some extent this may be true, data are available to show that differences may be seen among different ascites tumor lines[8] enough to make hazardous the reliance upon a single ascites tumor screen.

It has been fashionable to point out that we are trying to cure cancer in man and not in mice. It is necessary to recognize species differences, and for some this has led to the theoretical attractiveness of the human tumors grown in heterologous hosts. These are under active study, but not enough has been learned to indicate whether they possess any advantages to overcome some of the practical questions that have been raised concerning their utility.[6] As a matter of fact, all cancer chemotherapy screening studies suffer from the problem that there are no yardsticks, in the form of curative or remarkably effective drugs for the treatment of human cancer. A few such agents by which to challenge proposed screening procedures should permit concentration of effort upon more promising techniques. It is of interest to note, as we have previously,[6] that of the agents of limited but definite value in the clinic nearly all were first shown to be active against experimental animal tumors. Included in this group are: urethane,

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cortisone and hydrocortisone, methyl-bis (β-chloroethyl)amine (HN_2), CB 1348 (chlorambucil), Aminopterin and Methotrexate, TEM, myleran, TEPA, and thioTEPA, 6-mercaptopurine, 6-chloropurine and thioguanine. Exceptions are potassium arsenite, which was found useful many decades before animal cancers were available for experimental use, and the androgens and estrogens also found useful first in human cancer; in the case of these hormones there still are very few animal tumors suitable for demonstrating their activity.

With objections raised against the use of nonhuman, mammalian tumors, it may seem even more unreasonable to use microorganisms for screening. Though it would be surprising if such test objects accurately selected cancer chemotherapeutic agents in a large screening, the microorganisms may be quite helpful when utilized with more limited objectives. Use as a large prescreen has been mentioned. In addition, selected organisms may be used for search of certain antimetabolite activities as Hitchings[9] has done in studies for antipurines with *Lactobacillus casei* in special media. Another example lies in the guidance of the fractionations of antitumor culture filtrates of microorganisms by other microorganisms with specially detected susceptibilities to those antitumor principles even though the test organisms may show little general use in screening.

To a great extent, the test procedures used will be dictated by the characteristics of the test object. For example, some tumors that are rapidly growing may permit shorter test periods than those tumors that grow more slowly. Effects upon leukemias in mice may be judged readily by increases in survival time, whereas measurement of the weight or average diameter usually are best to determine the results of treatment in the case of a solid tumor. Differences in results can be anticipated based upon the experimental conditions adopted: for example with selected agents considerably different results, quantitative if not qualitative, may be seen based upon several choices in the routes of administration. Thus, some substances in which there is unusual interest, even if only on theoretical grounds, will require special handling. A routine screening cannot be expected to give each compound a maximum chance, or even an adequate opportunity, to show an activity. A greater resistance to agents has been observed among well-established tumors compared with the same tumor lines tested sooner after transplantation. It is unnecessary to point out to the statistically minded that the greater the uniformity in growth of test tumors the fewer will be the number of animals per test to yield a significant result. Also, it probably is unnecessary to point out that the closer a test procedure approaches the conditions of ultimate clinical use the more likely will be the chances of successful carry-over of results from experimental studies to the clinic.

The materials screened have included: many tested completely empirically, the concoctions from folklore, and numerous synthetic compounds tried for various reasons including some with a strong theoretical basis for choice. Chemically reactive compounds of many types have been tried, based to a large extent on the interest from the activity found with the chemically active nitrogen mustards. Many compounds have been tested upon the basis of some observed biologic activity, for example, antimitotic activity, as with colchicine; antimicrobial activity, as with 2,6-diaminopurine and 5-bromouracil, penicillin, and other

antibiotics. This in effect adopts the other biologic studies as prescreens to the extent that the actives from the other biologic tests have been tried in antitumor systems without necessarily rejecting the negatives from the other biologic test systems. Among the biologically active compounds on which most stress has been placed in recent years have been the antimetabolites. Compounds of potential antimetabolite activity have been studied regardless of lack of prior demonstrations of any biologic activity. It has been possible to find useful anticancer agents among the antimetabolites, for example, 6-mercaptopurine, and aminopterin, the 4-amino analog of folic acid. Although these have been useful agents, it is not surprising that their antagonism of early steps in nucleic acid synthesis causes limitations in usefulness through interference also with normal cell metabolism. It may well be that just as the ultimate indication is for test of materials in man so it is indicated that one must test materials more closely related to the ultimate difference in the gene or chromosome that characterizes a cancer cell. And while we must always challenge our test procedures and not be blindly satisfied with those we use, we must also keep before us the thought that our test procedures and test objects may not necessarily be defective but that we have merely failed to test the right materials.

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SUMMARY

At present cancer chemotherapy cannot boast of curative agents, but there are a number of drugs useful in the treatment of certain forms of malignancy in man. The large majority of these agents have first been detected as carcinostatic in experimental animal tumor studies. Thus, procedures capable of detecting activity do exist. There is a need to find, if possible, methods which are better because of greater reliability, less expense, and greater ease and speed in the attainment of information. A tremendous expansion in the national cancer chemotherapy program in the United States is increasing the testing with accepted methods while investigating the possible value of new procedures.

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CLINICAL INVESTIGATIONS IN CANCER CHEMOTHERAPY

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RATHER than attempt a summary of recent work in clinical investigations in cancer chemotherapy, it will be the purpose of this paper to illustrate a few basic principles of this discipline. These have been selected partly because they permit a few reflections on present trends and future directions, and partly because they emphasize for the reader the problems still to be solved.

One of the difficulties of the clinical investigator in this field is the risk to the patient accompanying the introduction of new potentially toxic chemicals. The first problem to be faced is the moral and ethical justification for such studies. Such justification may generally be had[1,2] when the following conditions are met: (1) that the senior investigator has obtained the informed consent of the patient or patient's family following a general explanation of the risks involved and the possible benefits; (2) that there have been adequate studies of the drug in experimental animals so that the limits of toxicity are defined and there is some possibility of antitumor activity in patients; (3) that the senior physician has had broad experience in drug trials; (4) that there exist facilities, staff, and attitudes which permit the most careful supervision of such trials in order that undue risk may be avoided; and (5) that sufficient attention has been paid to the design of the study so that there is a serious expectation of obtaining a definite answer to the question posed by the study. Since there is always some risk in the trial of new drugs in man, even when the first four conditions have been met, this risk can be justified only in terms of the importance of the information gained. If the experimental design is poor, no information can be obtained and therefore no risk justified. The clinical investigator, before he introduces new drugs into patients, has a strong obligation to consult with the biostatistician and the pharmacologist so that the conditions of the trial will assure the collection of useful and interpretable data.

The second problem in undertaking the administration of a new drug to man is how much to give and how to give it. In this problem there are three questions to be answered: (1) By what route shall the drug be administered? (2) What is the safe dose to start the trial? (3) With what dosage schedule should the drug be studied during the assessment of therapeutic effectiveness?

The proper route of administration can generally be settled in animal experiments. If a method is available for the determination of the drug in body fluids

the absorption and excretion of the drug can readily be examined in the dog and checked later in patients. Even in the absence of such a method, however, this information can be obtained by a consideration of the oral and intravenous doses required to produce equivalent toxicity in rodents or dog.

In determining a safe dose of a new drug for man, generally the investigator starts cautiously with a small dose, and after observing no toxicity gradually increases the dose. In order to arrive at an estimate as to the size of the original dose, it has generally been found safe to base this on the maximum tolerated dose in the most susceptible of several species of experimental animals. The major problem here revolves around the unfortunate fact that there are drugs which are much more toxic for man than for animals.[3-6] There are few drugs less toxic for man than for experimental animals. Experience has shown that adequate safeguards are available when one has studied the maximum tolerated dose both acutely and chronically for at least three species of animals. Generally, the mouse, rat, and dog are used, and it is wise to add monkeys, if available. One determines the maximum tolerated dose for a 2-week period for the most susceptible of these species. Trial in man is begun at one-tenth of this daily maximum tolerated dose on a milligram per kilogram basis. The drug is administered cautiously to man with stepwise increases (but never exceeding a duration of drug dosage longer than available chronic studies in animals) until mild side effects are obtained. From such studies it is possible to design a safe dosage for the period required in a therapeutic trial.

The third question of the optimal dosage schedule of the drug in the therapeutic trial is much more difficult to answer. Each drug is a law unto itself, and the optimal schedule[7] must be determined empirically. For example in the infectious disease field sulfonamides[8] must be given in such a way as to maintain a constant blood concentration whereas penicillin and terramycin can be given just as effectively without maintenance of the concentration in the blood.[9-11] Since drugs used in cancer chemotherapy are not often dramatically effective against clinical cancer, there is little information available on optimal dosage schedules of these drugs. Dosage schedules must hence be based upon experience in the experimental animal model. In such animal systems, Methotrexate in early leukemia in mice is most effective when given once every 4 days, but in far-advanced leukemia, it is most effective when given once daily.[12] How well this is true in man remains to be determined, but it is probable that a continuous blood concentration of Methotrexate is unnecessary in the treatment of human neoplasms.[13,14]

Before proceeding with the actual design of the therapeutic trial, it is necessary to answer several other questions. One of these is how long should a drug be administered to a patient with cancer so that there can be assurance that the drug has had time to act and that the tumor has had time to show regression? From experience with the rapid effect of radiation or alkylating agents on lymphomas, new antitumor drugs are sometimes administered for short periods of time, and the effect is called negative if no regression is seen within a few weeks. Experience with the antimetabolites, however, seems to show that often the response

of tumors to these agents is rather slow and gradual. For example, in acute leukemia substantial remission following the antifolic agents may not take place until 3 or 4 weeks have passed.[15] With 6-mercaptopurine[15] this lag may be as much as 5 to 8 weeks. Recent experience with the use of Methotrexate in choriocarcinoma[14] has shown that although the urinary output of chorionic gonadotrophins is rapidly reduced after starting drug, definite regressive changes in the pulmonary metastases may not appear until a month or more after the start of drug, and complete remission of the metastatic disease often requires two to three months. Even with the new alkylating agents such as Myleran[16] or Chlorambucil[17] significant effect on the susceptible tumors is often not well established until the third or fourth week. Certainly in the trial of the antimetabolite drugs, administration of the drug and observation of the patient for less than a month would probably not allow the drugs sufficient time to manifest their effects upon the tumor. Before calling a drug negative against a tumor in man, it would seem to me that one would need a series of patients treated for at least a month, and preferably for 6 weeks, with observations for somewhat longer periods. The same thing is probably also true of those alkylating agents which are administered on a daily basis instead of the intensive regimen used for nitrogen mustard.

Another question that must be answered is: What is the end point that the physician will choose to decide whether a drug has activity? At the present time there are four useful parameters. These are the histology of the tumor, the size of the tumor, the survival of the patient, and alterations in the output of chemical products produced by the tumor. The use of histology is probably most useful in the leukemias and those related diseases where it has been possible to induce complete hematologic remission by means of drugs. In the nonleukemic tumors, spontaneous necrosis is such a common finding that changes of this sort after drug administration have generally proved not to be useful. Changes in tumor size can be a most useful end point in determining drug effect if a serious effort is made to place this observation on a quantitative basis. Such a refinement in tumor measurement has recently been described by Brindley and his colleagues[18] wherein caliper measurements of the easily accessible tumors are graphed against time permitting the estimation of a slope which sensitively reflects reduction in tumor size. The use of clinical impressions of changes in tumor size without actual measurement is often misleading and is to be condemned. The same thing may be said if one attempts to study tumors such as intra-abdominal masses and intracranial tumors which are deeply situated and difficult or impossible to measure.

Calculation of survival time under certain circumstances can be made into a highly useful measure of therapeutic activity. This parameter is really useful only in the study of those tumors where the median survival time is something less than a year. Tumors which advance slowly and where the median survival time is a year or longer probably should not be studied in this fashion since the information accumulates too slowly to be of use in an active chemotherapy program. The use of life table analysis of survival is the most satisfactory way to compute median survival time.[19] One of the serious drawbacks to the use of this end point is the fact that survival time of many chronic diseases is gradually increasing because of general advances in medical knowledge. The utilization

of survival time data of untreated disease taken from even the fairly recent past is a base line upon which one can place little dependence. In order to interpret the effect of therapy upon survival time, one must be able to make a comparison with the survival of patients who have the same disease treated with modern methods but without the specific therapy under study. Many investigators now feel that studies of the effect of a drug on survival must be carried on concomitantly with the study of similar patients who are receiving standard accepted therapy, including chemotherapeutic agents known to be active. In this way significant differences in the prolongation of life brought about by a new drug may be firmly established by direct comparison with the effect of the best known therapy.

Finally, one may study the effect of a drug on the output of a chemical product by the tumor. If the drug is able to accomplish a sharp diminution of the outpouring of this chemical product from the tumor, it should be possible to use this as a measure of the effect of the drug against the tumor. So far, this has been systematically possible only with the chorionic gonadotrophins manufactured by choriocarcinoma. In other instances, such measures are occasionally useful. These include acid phosphatase in carcinoma of the prostate, abnormal proteins in multiple myeloma, and adrenal cortical steroids in adrenal cortical cancer. In summary, then, the only really broadly useful end points in the study of chemotherapeutic effects of antitumor agents are the caliper measures of tumor size in the nonleukemic tumors, the use of histologic changes in the leukemias, and the use of median survival times from life table analysis in the more fulminating neoplastic diseases.

The design of the therapeutic trial is determined by the specific purpose of the investigator in undertaking the trial. The possible purposes, in their most extreme forms, may be stated as follows: (1) to find a drug which quickly causes marked regression in a majority of patients with a wide variety of cancers; (2) to establish within 95 per cent confidence limits that a new drug is active against one kind of cancer; and (3) to define precisely the usefulness of an active drug in the treatment of one or more types of cancer in comparison with other active drugs, surgery, or radiation.

If the first purpose is the intent of the investigator, attention to the details mentioned earlier in the paper will suffice. This is the way in which the effect of nitrogen mustard was found,[20] when the first patient with Hodgkin's disease to receive it showed marked and immediate tumor regression. Unfortunately, many subsequent drug trials have imitiated this design. I say unfortunate because the limitations of this design are frequently not recognized. The only conclusion from such a trial is that a positive result indicates an active material. What can be concluded from a negative result with the first design? One may conclude only that the drug does not quickly cause marked regression in a majority of patients with a wide variety of cancers. Important to recognize in this connection is the number of patients with a single tumor type who must be given drug in order to find a therapeutic effect. With antifolic drugs which induce complete remission in at least 30 per cent of children with acute leukemia,[15] one group treated 12 children without noting any bone marrow remissions.[21]

Another observer saw no activity in the first 9 patients.[22] Whatever statistical theory would say, it is clear that in clinical practice a sample size of 10 patients with a single disease is barely adequate to obtain a positive result from drugs which can cause complete remission in 30 per cent of patients.

If the investigator wishes to define the usefulness of a new drug in comparison with standard therapy, as in the third purpose, then the principles of the classical therapeutic trial should be utilized.[23,24] These have been discussed elsewhere in relation to cancer.[25,26] By way of summary, such a trial includes careful definition of the kinds of patients to be studied and end points to be measured; unbiased randomization of patients between the two treatments; unprejudiced determination of therapeutic effects; statistical evaluation of data and cautious generalization. It is difficult if not impossible to undertake such studies in a single hospital; pooling of patients in 8 to 10 large hospitals is desirable so that the study can be completed in less than a year. The virtue of such studies is that they provide a sound basis for the utilization of therapeutic conclusions by the medical profession. Such a study can also tell the chemist and biologist about the correlation of drug effects in animals with therapeutic effects in man. However, as large numbers of new agents active in animal systems begin to appear, more rapid answers are highly desirable. This is expressed in the second purpose, and current attempts are being made to solve this problem by the use of sequential design techniques.[27] In this design, the investigator decides on what he is willing to accept by way of spontaneous remission of the disease under study and what risks he is willing to take in missing active compounds. It is then possible to treat a small series of patients with a new drug in comparison with other patients receiving a standard drug. As long as a defined number of successes are achieved, the study continues. If the successes exceed or fall below the critical level, the study is completed, for by definition the degree of activity has been determined. Correlation with the animal findings can be made and a decision quickly arrived at for the next step in the program. Such studies in cancer chemotherapy have just begun in this country,[28] and their worth will soon be known. Certainly some modification of this approach will be needed to examine the large number of drugs now in production.

The next question to be answered by the clinical investigator is what drugs shall he study? At the present time these are chosen in two ways. First, they are chosen in a "rational" manner by the use of biochemical knowledge in which the differential vulnerability of the cancer cell and the normal cell to various chemicals may be exploited. These chemicals are synthesized, tried in animal tumors, and, if active, studied in man. The second way of choosing drugs is by the empirical screening of whatever drugs are available in a series of animal tumors. Neither of these methods is satisfactory, and at the present time one of the main difficulties of a clinical investigator in cancer chemotherapy is his inability to utilize animal tumor systems and other screening techniques to achieve a higher degree of predictability for anticancer activity in man. The key to this difficulty, I believe, is that we have been collecting purely qualitative data on the activity of drugs, both in animal tumor systems and in patients with tumors. It is beginning to be recognized that in order to make full usage of the enormous resources which have been placed at the disposal of those studying cancer chemo-

therapy, it will be necessary to understand fully the correlation between the activity of drugs in experimental tumor systems with the effectiveness of drugs in patients with cancer. This can only be done by collecting quantitative data on activity in both. Only then will it be possible to compare the results with sufficient precision that the predictability value of the animal system for effectiveness against clinical cancer can be known. Such a quantitative animal model has been described by Goldin[29] and a national program on the collection of quantitative data in man is in progress.[30] Gradually the degrees of correlation between the two systems will be defined, and it will become possible for the clinical investigator to choose with some confidence the drugs to be studied in man.

Finally, the clinical investigator must decide what studies are needed in clinical pharmacology. The following needs of the clinician are presented.

1. The clinician needs the development of methods for the determination of drugs in biological fluids. With such methods it becomes possible to study rapidly the absorption, excretion, and distribution within the body of the drug under study. By their use the physician can learn how the drug gets into the body, how it stays there, and how it gets into the tumor and other tissues. The methods should preferably be chemical rather than those utilizing isotopic labels since it may be necessary to use these methods daily to determine blood concentrations in relation to efficacy. It may be possible in this fashion, as it was in the study of malaria,[31] to show that some drug failures may be due to the inability of the patient to maintain therapeutic concentrations of the drug when receiving standard dosages. Drug distribution studies are of importance to the clinician since for some drugs there will be no activity against the tumor unless the tumor is reached by the drug. For example, it has been shown that central nervous system leukemia[32] is unaffected even though a complete hematologic remission has been induced. Distribution studies have shown that the clearance of Methotrexate into the central nervous system is only about 10 per cent of plasma concentration.[32]

2. He needs methods for the study of the metabolism of drugs in man. The failure of some drugs which are active in animal tumor systems to be effective in man is explained by differential metabolism of the two species. For example, with riboflavin antagonists[33] the rat achieves high concentrations of an active metabolite while man converts the same drug into an inactive metabolite. The failure of animal tumor systems to predict activity for man may occasionally be explained on such differential metabolism. Another reason for studying drug metabolism in man is emphasized by recent data with regard to isoniazid in tuberculosis. Isoniazid is ineffective in man unless adequate amounts of the active metabolite are produced.[34] In these instances of variable degrees of drug metabolism, repeated observation of the capacity of the individual to attain sufficient quantities of active drug is needed.

3. He needs methods for the choice of the optimum dosage schedule of an active drug against tumors in man. This has been discussed earlier in the paper.

In conclusion, it is quite possible that clinical investigations in cancer chemotherapy are entering upon a new phase. The first phase was of qualitative nature, during which the magnificient contributions of relatively few investigators es-

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tablished that drugs could cause partial regression of a few cancers in man. The second and present phase is one in which the need for quantitative studies has been recognized. A national effort to uncover truly curative drugs is underway, in which the quantitative skills of the biologist, pharmacologist, biostatistician, and clinician are being utilized to filter out the clinically active materials from the endless products of the chemist. It is to be hoped that the correlations that can thus be established between the laboratory systems and the clinical effects will soon lead to the third and final phase in which many types of human cancer can be cured by drugs.

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SYMPOSIUM ON CHRONIC CONGESTIVE HEART FAILURE

GUEST EDITOR, DR. ALFRED P. FISHMAN

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SYMPOSIUM ON OCCUPATIONAL MEDICINE

GUEST EDITOR, LEONARD J. GOLDWATER, M.D.

To be published in a forthcoming issue of the JOURNAL OF CHRONIC DISEASES

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